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

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva

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

Online supplement

Homogeneity and quality of the study

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

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

Reasons for excluding patients

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

Table S1. Main reason for excluding adult patients from randomization

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

Anticoagulation protocol

Patient with no increased risk of bleeding:

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

Heparin-induced thrombocytopenia:

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

Patient with increased risk of bleeding:

Prostaglandins can be considered (grade E).

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

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

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

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

Patient with increased tendency to clot:

Add prostaglandins to UFH or LMWH (grade C):

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

Regional anticoagulation

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

Treatment schedule

Prefilter:

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

Postfilter:

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

Important advices:

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

Interim Analyses

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

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

Methods to develop the multivariate logistic regression model

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

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

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

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

Patients characteristics

Table S2. Characteristics of the patients before randomization

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

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

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

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

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

Sensitivity analyses

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

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

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

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

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

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

References

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