PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial.
AUTHORS	Livigni, Sergio; Rossi, Carlotta; Ferrari, Fiorenza; Giardino, Michele; Pozzato, Marco; Remuzzi, Giuseppe; Bertolini, Guido

VERSION 1 - REVIEW

REVIEWER	PAGE Mathieu. M.D. Department of Anesthesiology and Intensive Care, Edouard Herriot Hospital, Lyon, France I have no competing interests
REVIEW RETURNED	10-Aug-2013

GENERAL COMMENTS	Livigni et al. reports the results of the COMPACT study assessing effect of CPFA on hospital mortality and various secondary endpoints. This is an important study, including 330 ICU patients over 18 italian ICU. This is the most important study about CPFA. The study design is correct, primary and secondary endpoints are relevant. The manuscript is well written. Even if they are negative, the results of this study should be published because they add a stone to the knowledge about blood purification techniques and CPFA. The authors should be congratulated on their honesty to publish negative data on CPFA. We tend to learn more from our failures than successes. I have few comments - methods : if possible, the authors should use the 2013 KDIGO classification of acute kidney injury instead of RIFLE (depends on the data collected). - in methods, a short section describing the CPFA circuit with an additional figure should be usefull to the reader who is not familiar with the technique. - I believe methods section describing per protocol analysis is quite hard to understand. The problem seems to come from the sentence "On the other hand, there was no evidence that outcome in patients who received lower volume treatment was statistically better or worse than controls". The authors could clarify this point, either removing this sentence or by adding values of the control group in Figure 3. - important concept and hypothesis are exposed in the discussion to explain the negative results. However, the discussion could greatly be improved if it was more structurated and focused. I believe too much room is allocated to justifications of the results, the anticipated stop, the choice of the primary endpoint. The authors could bring together limitations of the study in a same section (2 or 3 main limitations), a section with a discussion of the important concepts

	such as the "plasma treatment dose" by extending the concept of "dialysis dose". And maybe the perspectives : whould we abandon CPFA ? should we study other strategies (other plasma rates, selected patients with the most critical conditions ?) - Some patients without AKI have been treated with this extracorporeal blood purification therapy. Should we consider proposing blood purification therapies only in sepsis associated with AKI ? In conclusion : interesting work, it should be published but could be improved with few revisions.

REVIEWER	PAOLO LENTINI M.D, PhD NEPHROLOGY AND DIALYSIS SAN BASSIANO HOSPITAL BASSANO DEL GRAPPA (VI) ITALY
REVIEW RETURNED	13-Aug-2013

RESULTS & CONCLUSIONS	48% of the patients don't reach the amount of plasma treatment required. CPfa is a plasma treatment with dose-depent effects For this reason CPFA results in this study will be re-analyzed in a larger sample of septic-critical ill.

REVIEWER	John C. Marshall MD Professor of Surgery University of Toronto, Canada No relevant competing interetss declared
REVIEW RETURNED	21-Aug-2013

RESULTS & CONCLUSIONS	the study is small and terminated permaturely, thus unable to answer the primary question regarding treatment efficacy
GENERAL COMMENTS	This manuscript reports a multicenter open label Italian RCT undertaken to test the hypothesis that coupled plasma filtration adsorption (CPFA) will improve the survival of patients admitted with a diagnosis of sepsis. The study was terminated prematurely for futility after 192 patients had been enrolled, although a pre-specified subgroup analysis suggested efficacy in patients who received high volume therapy. This is a well-designed study addressing a clinically relevant problem. The support of an experienced investigator-led research network – GiViTI – is a definite strength. The anticipated therapeutic signal is optimistically large given the complexity of the trial and the heterogeneity of the study population, however given this, the decision to terminate prematurely is understandable. On the other hand, premature termination precludes the detection of a small, but clinically important therapeutic signal. In essence, this study was, if not by design then by result, a pilot study testing the feasibility of the intervention, and its most important insights lie in this domain. The major shortcoming of the report is the effort to imply efficacy in a small study whose primary message is the challenges of conducting a rigourous RCT of a complex intervention.

	The paper requires copy editing to correct minor errors in grammar. In addition, I would ask the authors to address the following specific comments. Specific Comments 1. The biology of sepsis is complex, and so needs to be described cautiously, and consistent with what is known. Thus the statement in the first paragraph of the Introduction that, "… 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 …" is overly simplistic; a more nuanced description is needed. 2. How did you operationalize the timing of diagnosis of septic shock? Your protocol provides only a very narrow 6 hour window for patient recruitment and initiation of treatment, and so it is important to know how you ascertained when the 6 hour window should start. 3. Why did you exclude pregnant women? There seems to be no reason for anticipating fetal harm. 4. The method of randomization is unclear. It appears that some kind of randomization code is a part of the eCRF, however how this is operationalized is not stated. Is this a binary value applied to all patients in the database? And if so, how was it applied, and how do you control for the fact that most patients won't be eligible for the trial? How did the process guarantee concealment of allocation? 5. You excluded 8 patients post-randomization; even though they may have been entered erroneously, and intent-to-treat analysis should include these patients in the arm to which they were randomized. Please provide the results of this analysis, or justify a post-randomization (and therefore no longer random or truly intent-to-treat) alteration to the analytic plan. 6. The non-eligible patients had a higher mortality (thank you for providing these data). Can you provide insight into why they were excluded, since the higher mortality suggests a potentially greater possibility of response to intervention. 7. Why were the 88 patients who were eligible for inclusion not randomized? 8. Almost 50% of patients in the intervention arm failed to receive the intervention at the planned dose and intensity. What sort of education was performed to try to prevent this, and why was the non-compliance rate so high? 9. The post hoc analysis by intensity of treatment is very confusing. To whom does the statement, "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" apply? 10. Three patients in the control group were treated with CPFA, in violation of the protocol. What happened to the other 2 (1 day in the first 7 days post-randomization)?

	11. A “near significant trend” in a secondary outcome is unlikely to be a clinically important signal; please tone down your comments. 12. Given the small sample size, premature study termination, and lack of evident effect in the overall ITT population, the post hoc analyses suggesting the possibility of efficacy are overly optimistic and much more likely to be a function of random variability. Please focus the analysis on the impediments to the conduct of a trial, and how these might be overcome; the suggestion of treatment efficacy is really not supported, and a study such as this should not change practice, but rather inform the design of future work. 13. Your analysis raises the possibility that challenges with protocol compliance may have prevented a positive study signal. Please orient your discussion around issues of feasibility and design – what did you learn that could inform a more appropriate study – rather than around clinical efficacy, which is patently not supported in your trial. 14. It would seem that your primary challenges were not trial endpoints, but rather compliance with the intervention – both in providing the planned intensity of treatment and in preventing crossovers; what information have you gleaned that might address these issues and facilitate the design of future work to test the intervention? 15. Please discuss the limitations of your study in the Discussion.

REVIEWER	Frank Bloos, MD, Ph.D. ICU consultant Dept. of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Germany FB has no competing interests regarding the content of the reviewed manuscript.
REVIEW RETURNED	22-Aug-2013

THE STUDY	Supplemental documents: Information about the applied adsorption filter and basic information about the desired setup should be given in the manuscript.
RESULTS & CONCLUSIONS	Written consent from septic patients is usually not possible and lack of informed consent was an exclusion criterion. The authors state that GCP guidelines were applied when informed consent of the patient was not possible. What does this mean? How was inclusion process undertaken in patients not capable for informed consent.
GENERAL COMMENTS	Major comments ========== * Life expectancy of <2 weeks were assessed by the treating physician. This assessment affects the primary outcome parameter. This has the potential to introduce a severe selection bias since the study was unblinded. Thus, the treating physician can affect the outcome parameter by knowing the intervention. Was there a formal follow-up? The study assesses 90 day mortality rates. Why did you then leave the 2 weeks assessment to the discretion of the treating physician. * The study has a high number of protocol violations due to technical reasons. This has been addressed by the authors. However, its is

	therefore not possible to assess whether futility was caused by ineffectiveness of the CPFA or CPFA-undertreatment in the intervention group. This should be addressed in the conclusion. * The conclusion should also be rephrased because it introduces new information and discussion such as the immune status of the patient. Please, constrict the statements in the conclusion to your final assessment of the study results. * The study used the Project Margherita CRF as documentation tool for this study. This CRF was developed for an observational project. Thus, it is not a CRF specifically designed for the conduct of the presented randomized trial. Please, discuss. Minor comments ========== * page 4; lines 45-47: 'GiViTI, the ICU network...' state the effects on what (mortality) are investigated. * The definition of the per protocol set should be given in the statistics section * Page 8, line 43-55: 'We performed two sensitivity analyses...'. Please, describe the analyses in the Methods section and give only the results here. * Page 9, line 24: 'To overturn these results...' The meaning of this sentence is unclear.

REVIEWER	Dr Stephen-Mark Cooper Reader & Graduate Studies Coordinator Cardiff School of Sport Cardiff Metropolitan University Cardiff, Wales, UK
REVIEW RETURNED	03-Oct-2013

GENERAL COMMENTS	In this manuscript (MS), the authors have applied some relatively basic but nonetheless appropriate statistical methods to investigate what was an Italian multicentre randomized clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock - 18 general intensive care units with 184 adult patients. As I am not an expert on the body of research that is the focus of this MS I have limited myself to making general comments about the MS and its presentation, and more specifically the research design and the statistical analyses of the data. On the whole, the authors communicate their arguments in a clear manner. The paper is generally well-written and it is skilfully crafted. In my view, however, the paper is overlong, especially as the study was prematurely terminated ‘on the grounds of futility’ (p.6, line 44).

	Nonetheless, the paper will be useful to researchers and practitioners in considering the efficacy of CPFA. The study is well-designed, the research is well-organised, the data seem to have been collected appropriately and, even though the statistical methods employed are fairly basic, the data have been expertly handled and analysed. As far as the methodology is concerned I am convinced that I could replicate the study based on the description the authors provide in the MS. The Tables and Figures appended to this MS are extensive and the information contained therein is useful but is not always easy to interpret. I would describe them as 'busy'! If I have any criticisms of the MS at all it is to do with the lack of a good proof reading before submission as there are certain inconsistencies in the expression of terms and the way some variables are notated and the way units of measurement are expressed (eg l/kg/day should probably be L kg^{-1} d^{-1} or L kg^{-1} day^{-1}). A good mix of statistical methods is employed, including: χ^2 test, effect size, 95%*CI*s, Fisher's exact test, *t*-tests, Cochran-Armitage test and logistic regression. The only criterion for the choice of test mentioned in the MS however, seems to be based on whether variables were deemed categorical or continuous (p.7, lines 4-6). Nothing about assumptions underpinning these tests is mentioned – normality of residuals, homogeneity of variance as well as the level of measurement mentioned. The first paragraph on p.8 provides a good example of the inconsistencies in the expression of terms that I am concerned about. In line 10 the authors maintain that '... endpoints did not differ ...' when in fact they were numerically different. What the authors mean is that these endpoints did not differ statistically. Again, on line 14 '... also no differences in the *a priori* determined ...' they were numerically different but 'not significantly different'. This was an issue identified throughout the MS. I would also like to see all statistical indices expressed in italics where appropriate (e.g. *CI*, *P*, *n*, χ^2, etc.). Finally, in the Tables and Figures, sample numbers should be expressed as *n* not N. *N* = number in a population and, *n* = a sample size. I realise that such points might seem pedantic but then … one man's pedantry is another man's precision! Seriously, this was a really good paper, and even though I have no background in the academic

	area other than the research design and the statistics used, it was one which I thoroughly enjoyed reading. It was a pity that the authors had to prematurely terminate the trial. Nonetheless, it was appropriate that the authors identified in the MS that the 'nonbinding Bayesian futility criteria for stopping the trial were not fulfilled' (p.9, line 36).

VERSION 1 – AUTHOR RESPONSE

Reviewer: PAGE Mathieu. M.D.
Department of Anesthesiology and Intensive Care, Edouard Herriot Hospital, Lyon, France

I have no competing interests

Livigni et al. reports the results of the COMPACT study assessing effect of CPFA on hospital mortality and various secondary endpoints.
This is an important study, including 330 ICU patients over 18 Italian ICU. This is the most important study about CPFA.
The study design is correct, primary and secondary endpoints are relevant. The manuscript is well written.
Even if they are negative, the results of this study should be published because they add a stone to the knowledge about blood purification techniques and CPFA. The authors should be congratulated on their honesty to publish negative data on CPFA. We tend to learn more from our failures than successes.

Re: We sincerely thank the referee for this comment.

I have few comments
- methods: if possible, the authors should use the 2013 KDIGO classification of acute kidney injury instead of RIFLE (depends on the data collected).

Re: The definitions of acute kidney injury grade adopted by KIDGO are the same of AKIN, and different from RIFLE. The two are indeed very similar, apart from the inclusion of a GFR criteria in the RIFLE. We agree with the referee that the AKIN/KIDGO criteria are simpler, without losing specificity, and thus preferable, as also reported in the literature. For this reason, we have adopted the AKIN/KIDGO definitions in our ongoing "Margherita Project", starting from 2011. Unfortunately, in the COMPACT project we adopted the RIFLE criteria and are unable to extract from the data available the AKIN/KIDGO ones.

- in methods, a short section describing the CPFA circuit with an additional figure should be useful to the reader who is not familiar with the technique.

Re: We agree with the referee. We originally put this paragraph in the appendix for space constraint. We now moved it into the main text, as suggested.

- I believe methods section describing per protocol analysis is quite hard to understand. The problem seems to come from the sentence "On the other hand, there was no evidence that outcome in

patients who received lower volume treatment was statistically better or worse than controls". The authors could clarify this point, either removing this sentence or by adding values of the control group in Figure 3.

Re: We deleted the sentence

- important concept and hypothesis are exposed in the discussion to explain the negative results. However, the discussion could greatly be improved if it was more structured and focused. I believe too much room is allocated to justifications of the results, the anticipated stop, the choice of the primary endpoint. The authors could bring together limitations of the study in a same section (2 or 3 main limitations), a section with a discussion of the important concepts such as the "plasma treatment dose" by extending the concept of "dialysis dose". And maybe the perspectives: would we abandon CPFA ? should we study other strategies (other plasma rates, selected patients with the most critical conditions ?)

Re: We have now structured the discussion in paragraphs, added further considerations about the problem of under-treatment (in the homonymous paragraph) and a new paragraph discussing other study limitations. We also clarified our perspective about the use of CPFA at this stage (last sentence of the paragraph “The problem of under-treatment” and Conclusions).

- Some patients without AKI have been treated with this extracorporeal blood purification therapy. Should we consider proposing blood purification therapies only in sepsis associated with AKI ?

Re: The presence of AKI was not an inclusion/exclusion criterion of the study. Thus, both patients with and without AKI have been treated with CPFA.
The OR of CPFA was 0.86 (95%-CI, 0.32-2.31) in patient with AKI and 0.86 (0.41-1.80) in patients without AKI (Breslow-Day test for homogeneity of ORs, p=0.99). Thus, no statements can be made on this regards.

In conclusion: interesting work, it should be published but could be improved with few revisions.

Reviewer: PAOLO LENTINI M.D, PhD
NEPHROLOGY AND DIALYSIS
SAN BASSIANO HOSPITAL
BASSANO DEL GRAPPA (VI) ITALY

48% of the patients don't reach the amount of plasma treatment required.
CPFA is a plasma treatment with dose-dependent effects For this reason CPFA results in this study will be re-analyzed in a larger sample of septic-critical ill.

Re: We agree with the referee. The hypothesis of a dose-dependent effect is an interesting result of the COMPACT study. We have just started a new adaptive trial with the aim of studying whether: i) the new-generation machine will allow to easily reach the minimum volume of plasma to be treated to possibly see a survival improvement; ii) the achievement of such a target is associated to an intermediate clinical outcome (i.e., healing from the septic shock); iii) the achievement of such a target is associated to a final improvement in survival.

Reviewer: John C. Marshall MD
Professor of Surgery
University of Toronto,
Canada

No relevant competing interests declared

General Comments

This manuscript reports a multicenter open label Italian RCT undertaken to test the hypothesis that coupled plasma filtration adsorption (CPFA) will improve the survival of patients admitted with a diagnosis of sepsis. The study was terminated prematurely for futility after 192 patients had been enrolled, although a pre-specified subgroup analysis suggested efficacy in patients who received high volume therapy.

This is a well-designed study addressing a clinically relevant problem. The support of an experienced investigator-led research network – GiViTI – is a definite strength. The anticipated therapeutic signal is optimistically large given the complexity of the trial and the heterogeneity of the study population, however given this, the decision to terminate prematurely is understandable. On the other hand, premature termination precludes the detection of a small, but clinically important therapeutic signal. In essence, this study was, if not by design then by result, a pilot study testing the feasibility of the intervention, and its most important insights lie in this domain. The major shortcoming of the report is the effort to imply efficacy in a small study whose primary message is the challenges of conducting a rigorous RCT of a complex intervention.

Re: We fully agree with the referee, and changed the discussion to further clarify this interpretation.

The paper requires copy editing to correct minor errors in grammar. In addition, I would ask the authors to address the following specific comments.

Specific Comments

1. The biology of sepsis is complex, and so needs to be described cautiously, and consistent with what is known. Thus the statement in the first paragraph of the Introduction that, "… 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 …" is overly simplistic; a more nuanced description is needed.

Re: We agree with this comment. Indeed, after many corrections and shortening of the original version, the final wording was eventually unsatisfactory. We have changed the first paragraph accordingly.

2. How did you operationalize the timing of diagnosis of septic shock? Your protocol provides only a very narrow 6 hour window for patient recruitment and initiation of treatment, and so it is important to know how you ascertained when the 6 hour window should start.
Re: This very narrow window was indeed one of the main reason for excluding patients from the study. We were so strict in order to be consistent with the physiopathological rational of the treatment. In this framework, the 6-hour window started from the onset of the hypotension refractory to fluids resuscitation. We specified this criteria in the text.

3. Why did you exclude pregnant women? There seems to be no reason for anticipating fetal harm.

Re: It was only a prudential criterion. However, no patient was excluded for this reason.

4. The method of randomization is unclear. It appears that some kind of randomization code is a part of the eCRF, however how this is operationalized is not stated. Is this a binary value applied to all patients in the database? And if so, how was it applied, and how do you control for the fact that most patients won't be eligible for the trial? How did the process guarantee concealment of allocation?

Re: A randomization process was indeed included in the eCRF and automatically launched in real time only after the patient was enrolled. Thus, the process didn't work for non-eligible patients. It is to be noted that, since a blocked randomization schedule based on the presence of septic shock on admission was applied, the randomization process could only be execute real time.
With two strata per center, and by randomly permuting blocks of four and six, it was virtually impossible to predict the allocation of the next patient. Furthermore, 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 the allocation concealment.
We better clarified this in the text.

5. You excluded 8 patients post-randomization; even though they may have been entered erroneously, an intent-to-treat analysis should include these patients in the arm to which they were randomized. Please provide the results of this analysis, or justify a post-randomization (and therefore no longer random or truly intent-to-treat) alteration to the analytic plan.

Re: We respectfully disagree with the reviewer on this point. As Dean Fergusson, Gordon Guyatt, et al. clarified (Postrandomisation exclusions: the intention to treat principle and excluding patients from analysis, BMJ 2002;325:652–4): "Patients may be inappropriately randomised into clinical trials as a result of human error. Many clinical trials involve acutely ill patients who require urgent interventions. Determination of patients' eligibility for inclusion in these studies must be made quickly and consent and randomisation arranged expediently. Often study personnel work in chaotic clinical environments. Time constraints may result in patients who do not meet predetermined eligibility criteria being mistakenly included."
This is what happened in our study for those 8 patients.

Again: "When ineligible patients are mistakenly included, investigators could remove these patients from both study arms without risking bias. However, so that the decision to remove such patients is unbiased and not influenced by events that occurred after randomization (and may therefore be affected by whether patients received experimental or control treatment), an independent adjudication committee blinded to treatment and outcome must systematically review each patient."
This is what we did. We identified 14 patients for whom the eligibility criteria were in doubt and submitted them to the EDSMC. The external committee determined that 8 of these patients were erroneously enrolled as they did not meet inclusion criteria. Only these patients were excluded from the analyses.

Finally: "If the reason for exclusion was that they were expected to have a reduced or no response to treatment, and the expectation is correct, their inclusion will introduce random error and reduce the power of the study and the precision of the estimate of treatment
effect."
This is our case since, as detailed in the online supplement, four patients were terminally ill, one had cerebral coma and life expectancy less than two weeks (i.e., two exclusion criteria), and three did not

have septic shock. Thus, there was no reasons why these patients could respond to CPFA. For this reason we excluded these patients form both study arms. This does not affect the intention-to-treat principle, which applies to truly eligible patients.
We now provide more details about these patients in the online supplement.

6. The non-eligible patients had a higher mortality (thank you for providing these data). Can you provide insight into why they were excluded, since the higher mortality suggests a potentially greater possibility of response to intervention.

Re: We provided the detailed reasons for excluding these patients in the online supplement

7. Why were the 88 patients who were eligible for inclusion not randomized?

Re: In most cases the reason was the impossibility to start the treatment within 6 hours from the occurrence of septic shock (52 cases due to the timing of diagnosis, 22 for organizational problems, 5 for technical problems). In 9 cases the patient had been already included in another study, which was not an exclusion criteria.
We added these specifications to figure 2.

8. Almost 50% of patients in the intervention arm failed to receive the intervention at the planned dose and intensity. What sort of education was performed to try to prevent this, and why was the non-compliance rate so high?

Re: We put the maximum effort we were capable of in training the centers. It is described in details in the online supplement, where we have now added some further information.
It is honestly very difficult to answer the last question. We can assure it was frustrating to observe such a high rate of failure in reaching the target volume of plasma to be treated, after all this effort. This put all of us (researchers and even investigators!) in check. Surely the procedure was a complex one. Furthermore, because of its high cost, in most cases the physicians did not start a new treatment in the same day, after the first clotting of the circuit. But we have also to admit that the training could have been at least partly ineffective. On the one hand it only reached a few persons 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 we cannot a posteriori exclude it was qualitatively suboptimal, even though we are sincerely not able to figure out why, above all after having received excellent feedbacks from participants.
We added these considerations in the text.

9. The post hoc analysis by intensity of treatment is very confusing. To whom does the statement, “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” apply?

Re: Those numbers apply to all the 91 patients randomized in the CPFA arm, as is now specified in the text.

10. Three patients in the control group were treated with CPFA, in violation of the protocol. What happened to the other 2 (1 died in the first 7 days post-randomization)?

Re: There was a typo in the text. Only two control patients actually received CPFA, in violation of the protocol. One died at 7 days post-randomization, the other was discharged alive from the hospital 37 days after randomization. We added this info in the text.

11. A “near significant trend” in a secondary outcome is unlikely to be a clinically important signal; please tone down your comments.

Re: We have corrected the sentence accordingly.

12. Given the small sample size, premature study termination, and lack of evident effect in the overall ITT population, the post hoc analyses suggesting the possibility of efficacy are overly optimistic and much more likely to be a function of random variability. Please focus the analysis on the impediments to the conduct of a trial, and how these might be overcome; the suggestion of treatment efficacy is really not supported, and a study such as this should not change practice, but rather inform the design of future work.

Re: We didn’t absolutely intend to be supportive of CPFA on the basis of the subgroup analysis. We have now added in the conclusion a clear message that, given the present results, CPFA should not be used in everyday practice. We also better explained in the conclusion that, in our opinion, the rational for a new confirmatory trial would hold only once the possibility to easy reach high volume of plasma treated through the citrate regional anticoagulation has been confirmed.

13. Your analysis raises the possibility that challenges with protocol compliance may have prevented a positive study signal. Please orient your discussion around issues of feasibility and design – what did you learn that could inform a more appropriate study – rather than around clinical efficacy, which is patently not supported in your trial.

Re: We have now discussed in more details the feasibility problems in “The problem of under-treatment” paragraph of the discussion.

14. It would seem that your primary challenges were not trial endpoints, but rather compliance with the intervention – both in providing the planned intensity of treatment and in preventing crossovers; what information have you gleaned that might address these issues and facilitate the design of future work to test the intervention?

Re: We do think that, with the actual machine, that only supports heparin anticoagulation, a new trial would hardly obtain different outcome. And even if this eventually happened, it would be the result of such a great use of resources that it would not be reproducible within clinical practice. For this reason, we believe the problem is not so much to increase the compliance of a technique that already demonstrated to be hardly feasible within a RCT, as to have available a new technique that overcomes the problem of the clotting of the circuit. Only the availability of such a novel technical apparatus would make a new trial reasonable.
We tried to better explain our perspective in the conclusion.

15. Please discuss the limitations of your study in the Discussion.

Re: We added a paragraph discussing other limitations of the study, besides the ones already

discussed in the text (i.e., under treatment, subgroup analysis).

Reviewer: Frank Bloos, MD, Ph.D.
ICU consultant
Dept. of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Germany

FB has no competing interests regarding the content of the reviewed manuscript.

Supplemental documents:
Information about the applied adsorption filter and basic information about the desired setup should be given in the manuscript.

Re: We agree with the reviewer about this comment, that was also made by Dr. Page. We originally put this paragraph in the appendix for space constraint. We now moved it into the main text, as suggested.

Written consent from septic patients is usually not possible and lack of informed consent was an exclusion criterion. The authors state that GCP guidelines were applied when informed consent of the patient was not possible. What does this mean? How was inclusion process undertaken in patients not capable for informed consent.

Re: We referred to the article 4.8.15 of the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice E6(R1):
“4.8.15. In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subject’s legally acceptable representative is not available, enrolment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favourable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject's legally acceptable representative should be informed about the trial as soon as possible and consent to continue and other consent as appropriate (see 4.8.10) should be requested.”
We now specify this in the text

Major comments
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* Life expectancy of <2 weeks were assessed by the treating physician. This assessment affects the primary outcome parameter. This has the potential to introduce a severe selection bias since the study was unblinded. Thus, the treating physician can affect the outcome parameter by knowing the intervention. Was there a formal follow-up? The study assesses 90 day mortality rates. Why did you then leave the 2 weeks assessment to the discretion of the treating physician.

Re: The exclusion criteria were assessed before having randomized the patient. Thus, the attending physician could not have differentially affected the outcome by knowing the intervention.
There was a formal follow-up after 90 days from randomization; there was no assessment after 2 weeks. The 2-week life expectancy criterion was the estimated prognosis made by attending physician prior to randomize the patient. If the prognosis was so bad, the patient was excluded from the study and not randomized.
The rationale of such an exclusion criterion was that we did not think CPFA could have an impact on terminally ill patients.

We better explained that the exclusion criteria were assessed before having randomized the patient.

* The study has a high number of protocol violations due to technical reasons. This has been addressed by the authors. However, it is therefore not possible to assess whether futility was caused by ineffectiveness of the CPFA or CPFA-undertreatment in the intervention group. This should be addressed in the conclusion.

Re: We clarified that we were not able to discern whether the reason of the negative result was the lack of effectiveness (mainly due to widespread feasibility problems) rather than the lack of true efficacy, and discussed this more extensively.

* The conclusion should also be rephrased because it introduces new information and discussion such as the immune status of the patient. Please, constrict the statements in the conclusion to your final assessment of the study results.

Re: We rephrased the conclusion and moved the sentence about the immune status of the patients to another part of the discussion.

* The study used the Project Margherita CRF as documentation tool for this study. This CRF was developed for an observational project. Thus, it is not a CRF specifically designed for the conduct of the presented randomized trial. Please, discuss.

Re: This is not true. The Project Margherita eCRF is a multi-purpose tool, and it has been developed also to perform RCT in compliance with GCP.

Minor comments
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* page 4; lines 45-47: 'GiViTI, the ICU network...' state the effects on what (mortality) are investigated.

Re: Ok, done

* The definition of the per protocol set should be given in the statistics section

Re: Ok, done

* Page 8, line 43-55: 'We performed two sensitivity analyses...'. Please, describe the analyses in the Methods section and give only the results here.

Re: The sensitivity analyses directly descended from the findings of the per-protocol analysis. They are difficult to be anticipated in the methods section, where we described the analyses planned a priori.

* Page 9, line 24: 'To overturn these results...' The meaning of this sentence is unclear.

Re: We rephrased the sentence.

Reviewer: Dr Stephen-Mark Cooper
Reader & Graduate Studies Coordinator
Cardiff School of Sport
Cardiff Metropolitan University
Cardiff, Wales, UK

In this manuscript (MS), the authors have applied some relatively basic but nonetheless appropriate statistical methods to investigate what was an Italian multicentre randomized clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock - 18 general intensive care units with 184 adult patients.

As I am not an expert on the body of research that is the focus of this MS I have limited myself to making general comments about the MS and its presentation, and more specifically the research design and the statistical analyses of the data.

On the whole, the authors communicate their arguments in a clear manner. The paper is generally well-written and it is skilfully crafted. In my view, however, the paper is overlong, especially as the study was prematurely terminated 'on the grounds of futility' (p.6, line 44). Nonetheless, the paper will be useful to researchers and practitioners in considering the efficacy of CPFA.

The study is well-designed, the research is well-organised, the data seem to have been collected appropriately and, even though the statistical methods employed are fairly basic, the data have been expertly handled and analysed. As far as the methodology is concerned I am convinced that I could replicate the study based on the description the authors provide in the MS.

The Tables and Figures appended to this MS are extensive and the information contained therein is useful but is not always easy to interpret. I would describe them as 'busy'!

If I have any criticisms of the MS at all it is to do with the lack of a good proof reading before submission as there are certain inconsistencies in the expression of terms and the way some variables are notated and the way units of measurement are expressed (eg l/kg/day should probably be L kg-1 d-1 or L kg-1 day-1).

Re: We sincerely thank the referee for these comments. (S)he is right that the statistical methods employed here are quite basic. But, after all, in a RCT much of the work is done by design (randomization, concealment, etc.) rather than by analysis.
The notations were ashamedly wrong. Thanks for correcting.

A good mix of statistical methods is employed, including: $\chi 2$ test, effect size, 95%CIs, Fisher's exact test, t-tests, Cochran-Armitage test and logistic regression. The only criterion for the choice of test mentioned in the MS however, seems to be based on whether variables were deemed categorical or continuous (p.7, lines 4-6). Nothing about assumptions underpinning these tests is mentioned – normality of residuals, homogeneity of variance as well as the level of measurement mentioned.

Re: We have specified how the assumption of the t test were assessed. The logistic regression model building strategy, along with the assumptions' evaluation, are detailed in the online supplement

The first paragraph on p.8 provides a good example of the inconsistencies in the expression of terms

that I am concerned about. In line 10 the authors maintain that '... endpoints did not differ...' when in fact they were numerically different. What the authors mean is that these endpoints did not differ statistically. Again, on line 14 '... also no differences in the a priori determined...' they were numerically different but 'not significantly different'. This was an issue identified throughout the MS. I would also like to see all statistical indices expressed in italics where appropriate (e.g. CI, P, n, $\chi 2$, etc.). Finally, in the Tables and Figures, sample numbers should be expressed as n not N. N = number in a population and, n = a sample size.

Re: We have corrected all these mistakes, thanks.

I realise that such points might seem pedantic but then … one man's pedantry is another man's precision! Seriously, this was a really good paper, and even though I have no background in the academic area other than the research design and the statistics used, it was one which I thoroughly enjoyed reading. It was a pity that the authors had to prematurely terminate the trial. Nonetheless, it was appropriate that the authors identified in the MS that the 'nonbinding Bayesian futility criteria for stopping the trial were not fulfilled' (p.9, line 36).

Re: We again sincerely thank the referee for these comments.

VERSION 2 – REVIEW

REVIEWER	John Marshall Professor of Surgery University of Toronto St. Michael's Hospital Toronto CANADA
REVIEW RETURNED	25-Nov-2013

THE STUDY	This is a revised version of a clinical trial report previously reviewed for BMJ Open. The authors have provided strong responses to issues raised by myself and the other reviewers, and I am pleased with their replies and the revisions made which have strengthened the paper.

REVIEWER	Frank Bloos Jena University Hospital Dept. of Anaesthesiology and Intesive Care Medicine GERMANY
REVIEW RETURNED	25-Nov-2013

GENERAL COMMENTS	The authors have sufficiently revised the manuscript. Some minor comments remain: * Abstract: The CPFA-device name and company should also be named in the abstract. Line 3: "... (CPFA), removing inflammatory..." better: "... (CPFA) to remove inflammatory...". Last paragraph: "...reduce mortality, if..." better: "...reduce mortality, when ..." * Table 3 is only mentioned in the text. Is there any important difference to be named? I think the table should be moved to the electronic supplement.

	* The conclusion should be shortened and should not open a new discussion or contain references.

VERSION 2 – AUTHOR RESPONSE

Reviewer Name Frank Bloos
Institution and Country Jena University Hospital
Dept. of Anaesthesiology and Intesive Care Medicine GERMANY
Please state any competing interests or state 'None declared': None declared

The authors have sufficiently revised the manuscript. Some minor comments remain:

* Abstract: The CPFA-device name and company should also be named in the abstract. Line 3: "... (CPFA), removing inflammatory..." better: "... (CPFA) to remove inflammatory...". Last paragraph: "...reduce mortality, if..." better: "...reduce mortality, when ..."

RE: we corrected the text accordingly

* Table 3 is only mentioned in the text. Is there any important difference to be named? I think the table should be moved to the electronic supplement.

RE: we moved table 3 to the electronic supplement

* The conclusion should be shortened and should not open a new discussion or contain references.

RE: you are definitely right. We moved part of the conclusion into the main discussion.

Reviewer Name John Marshall
Institution and Country Professor of Surgery
University of Toronto
St. Michael's Hospital
Toronto
CANADA
Please state any competing interests or state 'None declared': None delared

This is a revised version of a clinical trial report previously reviewed for BMJ Open.

The authors have provided strong responses to issues raised by myself and the other reviewers, and I am pleased with their replies and the revisions made which have strengthened the paper.

RE: thank you indeed