Author's response to reviews

Title: Administration of fibrinogen concentrate for refractory bleeding in massively transfused, non-trauma patients with coagulopathy A retrospective study with comparator group

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Author's response to reviews: see over
Dear Dr Vincent,

Thank you very much for reviewing our manuscript and allow us to send you a revised version. We have answered point-by-point each reviewer’s comment. Overall, we think that the revision process has improved a lot our manuscript and we hope that it can reach enough priority to be published. Please, note that the manuscript has been rewritten completely in line with the suggestions of the reviewers and the editor.

Please, don’t hesitate to contact me if any doubt should arise.

Best Regards

Sincerely,

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Reviewer 1

Please note that the manuscript has been rewritten completely in line with the suggestions of the reviewers and the editor.

However, the comparison between groups should be reduced substantially if not removed all together since these results not are of major interest given that two completely different group of patients are compared. Those receiving fibrinogen concentrate are sicker, have more severe hemorrhage, have microvascular bleeding is not responding to the first transfusion package, also receives PCC, are more coagulopathic which precludes a meaningful comparison. The authors acknowledge this in the discussion but I suggest that the comparator is removed.

Response.
Many factors could influence the decrease in the total number of units transfused after administration of fibrinogen concentrate (FBNc). Therefore, a non-adjusted analysis is clearly insufficient to document the effect of FBNc on the overall reduction of the number of transfused units. Recent systematic reviews from the Journal and Cochrane Library (please, see references 1, 3, 6 on the manuscript) have stressed the importance of including a group comparator when considering the effectiveness of FBNc. In our study, we have provided a comparator group. As pointed by the reviewer, however, there are important differences between the comparator group and the study group. We have tried to diminish these differences through an adjusted analysis, and including a matching analysis, by which more homogenous groups were obtained. We have modified our statistical analysis including an important variable: to reach or not a final fibrinogen concentration \( \geq 2.0 \text{ g/l} \), as recommended by guidelines. The misbalance between groups is now more clearly acknowledged in the “study limitations”.

**Reviewer 2**

*Please note that the manuscript has been rewritten completely in line with the suggestions of the reviewers and the editor.*

This manuscript describes an important therapeutic intervention in clinical reality: efficacy of fibrinogen concentrate (FBNc) in severe bleeding. The results show that a small, non-individualized “one size fits all” strategy of “adjuvant” administrating only 2g FBNc rather late in ongoing bleeding as an “ultimo ratio” fails.

Response

Indeed, this is the conclusion of our manuscript: late administration of low doses of FBNc don't influence the global number of units transfused. However, the authors believe that the administered dose was not "one size fits all". The FBNc dose was 2 [2, 4] g, indicating that 25% of the patients received doses \( \geq 4 \text{ g} \) and another 25% received doses \( \leq 2 \text{ g} \). In our Centre, and perhaps in other centres, administration of FBNc is adjuvant, being only administered when therapy with blood components has failed. Our article is one of the few that underlines the importance of managing FBNc earlier and at higher doses. We believe that this message is important to avoid late and insufficient administration of FBNc.

*Importantly this is not a proof of inefficacy of FBNc per se but of its use. Although already acknowledged in some sentences in the manuscript this should be the main important and clear message in the paper.*

Response

We have emphasized this message in the conclusions of the new version of the manuscript (please, see page 14).

*Checking for reaching the treatment goal (fibrinogen level above 1.5-2 g/l) should be encouraged. From a rational perspective, it is not only an action (giving 2 g FBNc) that differentiates potentially patient outcome but it must rather be the achievement of a treatment goal (optimizing fibrinogen concentration above 1.5 – 2 g/l). Groups must be categorized according to this difference which also implies that even though patients*
received FBNc but still had too low fibrinogen concentrations, they are still in the “not optimized group”. The authors report that only 75% of patients achieved fibrinogen levels above 1.5 – 2 g/l after FBNc administration. Accordingly, the 25% without reaching the treatment goal need to be re-grouped out of the “fibrinogen group” and data need to be re-analysed.

Response

A significant change in fibrinogen levels and FIBTEM measurements might not be detected, even 24 hours after the administration of high doses of plasma or fibrinogen. In trauma patients, despite the administration of high doses of FBNc (median 6 g), the mean fibrinogen plasma level was not within range until 24 hours after its administration (Schöchl H Crit Care 2010). Massive plasma transfusion does not improve ROTEM values after 1 day from its administration (Khan S, J Trauma 2014;76:561). So, fibrinogen may reach an optimized range (≥ 1.5 - 2 g/l) lately.

According with the suggestion of the reviewer, we have also categorized the patients according to whether they reached or not an optimal fibrinogen level within a 24-hours period after MTP activation (table 5). Once again, not important differences were found with respect to our initial classification; i.e., only fibrinogen level on admission was associated with reaching a target fibrinogen level ≥ 2 g/l. We have not perform a matching process with respect to optimized fibrinogen level, owing to both groups presented similar SOFA (please, see table 5).

Please, see the new version of the manuscript for more details.

ROTEM is listed and FITEM is mentioned in the algorithm on the very last page. Please add ROTEM FIBTEM data before and after FBNc administration. You may consider re-grouping according to your defined treatment goal in that algorithm

Response

During the acute phase of bleeding, ROTEM FIBTEM was measured in less than 5% of patients. Therefore, this variable has not been considered in the analysis. For this reason, ROTEM measurement “when available” is clearly denoted in the algorithm of Figure 1. Therefore, it is not possible to re-group patients on the basis of that criterion.

Reading only the abstract shows inconsistent interpretation of the results: there it is reported that FBNc reduced transfusions from 6 to 3 but in the conclusion it is stated that there is no association with transfusion reductions. Please clarify.

Response

The non-adjusted analysis showed that FBNc administration decreased the total number of units transfused. However, this was not confirmed by the adjusted analysis. The abstract has been rewritten in a more understandable way.
You mention that FBNc contains 10 times more – compared to what?

Response
FBNC contains tenfold more fibrinogen than a standard unit of plasma. We have added this sentence in page 3.

You mention thrombelastography but according to the algorithm rather use ROTEM. Please give both viscoelastic tests throughout (thrombelastography TEG/thromboelastometry ROTEM) or specify according to the citation / what you really used.

Response
We have specified this according to the citation throughout the manuscript.

You cite reference #7 in the context of no labelling of FBNc as “adjuvant” therapy in MTP. You may add content from reference #13 on further information on existing labelling.

Response
We have incorporated the information from reference #13, as suggested by the reviewer, and changed the sentence. Please, note that reference 13 is now reference 14.

You state that evidence is scarce especially concerning a comparator group. Although point-of-care (POC) testing was the focus (rather than simply administration of FBNc or not) in the review by Bolliger and Tanaka, Transfus Med Rev 2013, you may add this piece of evidence and the 12 included trials.

Response
We have included this reference (reference 15). However, as you stated, the review of Bollinger and Tanaka was aimed to assess the effects of point-of-care use on transfusion and coagulation factors requirements, rather than the efficacy of FBNc for improving haemostasis and decreasing blood transfusion. Their conclusions were mainly based on the retrospective Görlinger study, which included only 10% of cardiac surgery patients needing massive transfusion. Interestingly, Bollinger and Tanaka concluded that the evidence of point-of-care use for improving clinical outcome is limited.

Methods:
Why were deaths in 24h excluded? How many patients died? Please include a flow chart of processes and numbers. If you exclude in the revision dead patients this must be clearly listed in the limitation section.

Response
In fact, only the patients who died shortly after the onset of overwhelming bleeding were excluded from the analysis, because we considered that FBNc and/or blood components administration might be of limited efficacy in this clinical setting. A flow chart has been added (please, see figure 2).
What is meant by “admission”? To the OR, ICU, study inclusion? Please clarify.

Response
We have clarified this statement in method section (page 6)

What is meant by “in overtransfused patients”? Hb-level above goal ranges? Please clarify.

Response
We have changed overtransfused by “massively transfused” throughout the manuscript

As pointed out above in the general comment, matching of patients can only be based on whether or not the therapeutic goal of sufficient fibrinogen levels have been reached or not. Without such an adequate grouping, any further complex statistical analyses of within-group comparisons and co-variables and intentions to compensate for severe selection bias are useless and misleading. Power analyses must also be based on this categorization according to the treatment goal and not the action of FBNc infusion. Why was the difference of 2 +/- 2 been chosen?

Response
As stated in the methods section, in this retrospective study, we evaluated the efficacy of FBNc administration in patients who had already received the first pack of massive transfusion. This is common practice in our centre, and it is precisely our own practice what we are evaluating.

We administer 2 [2, 4] g FBNc (roughly 25 mg/kg), which is at the lower limit of the dose recommended in the European Guidelines for Perioperative bleeding (2C). We have found that the late administration of low doses of FBNc did not influence the number of transfused units. As a result of our findings, we are now administering FBNc earlier and at higher doses.

In the new version of the manuscript, we have regrouped the patients according to whether they reached or not, a fibrinogen level $\geq 2$ g/l after MTP activation. The results from this analysis are still supporting our previous conclusions. Also, we clearly established the importance of achieving adequate levels of fibrinogen through a scheme-guided algorithm (see manuscript). We believe that the influence of FBNc administration of reaching or not optimal levels of fibrinogen should be elucidated by a RCT, rather than by a retrospective study.

The sample size was established by the statistician through the appropriate formulas, and $\pm 2$ is the standard deviation.

Results:
Changes according to re-grouping and re-analyses.

Discussion:
Changes according to re-grouping and re-analyses.

Response
Both analyses (grouping the patients according to either giving or not FBNc and
reaching or not optimal fibrinogen levels) suggest that low and late fibrinogen administration is useless for decreasing the number of transfused blood units. We have re-grouped and re-analyzed our patient sample, and no substantial changes were found. (please, see abstract, table 5 and comments thorough the manuscript)

p. 11:
Treatment goals in obstetric bleeding may be different compared to trauma bleeding (higher fibrinogen levels). Please correct or amend.

Response
We have deleted this statement in the new version of the manuscript

The European Trauma Guidelines (reference #14) recommend plasma OR a concentrate-based approach which is a diplomatic wording that fits to several treatment strategies. Please acknowledge. The ESA severe bleeding management guidelines (reference #10) in fact to not comment on a specific plasma:RBC transfusion ratio but it should be clearly mentioned that this European guideline definitely recommends preference of a POC-guided algorithm in the elective surgical settings.

Response
These statements are clearly acknowledged in the new version of the manuscript (please, see page 12)

p. 12:
Most current guidelines recommend to go for a fibrinogen concentration of 1.5 – 2 g/l (not 1.0 to 1.5). Please correct or amend.

Response
We have corrected this threshold (please, see page 12)

Lack of national licensing of FBNc is not necessarily linked to risk-benefit analyses. In fact RCTs, observational reports, pharmacovigilance data and animal experiments indicate a very high safety of FBNc (which is in contrast to PCC or rFVIIa). The respective 2 sentences in the discussion need clarification.

Response
We acknowledge these issues both in the introduction and the discussion sections.

Inversely and independent correlation between fibrinogen concentration and transfusion requirements is probably another main message in this paper (fig. 2). An increase by 4 units RBC per 1 g/l decrease in fibrinogen concentration (tab. 4) is a clinically highly relevant message.

Response
This had been already acknowledged in the old version of the manuscript. Nevertheless, this fact is underlined in conclusion section, key messages and the
new figure 3 (please, see pages 12, 15 and figure 3)

p. 13:
You may add to reference #6 also the analysis of Bolliger and Tanaka mentioned above on POC-guided algorithms plus FBNc within some algorithms.

Response
We have added and commented the paper of Bollinger and Tanaka (please, see reference 15, page 18 and introduction section, page 4)

ROTEM should be added whenever thromboelastography is mentioned because many POC-users in fact use ROTEM. The authors may consider mentioning in the paper that typical overall TEG and ROTEM tests (kaolin activated, EXTEM, INTEM) do not permit assessment (differential diagnosis) of functional fibrinogen but only the specific tests of “functional fibrinogen” in TEG and “FIBTEM” in ROTEM. So only “to run TEG or ROTEM” is also potentially insufficient but it requires clinicians to look for maximum clot firmness or A10 in the specific tests to indicate FBNc appropriately.

Response
As we used ROTEM only in a few patients, specific tests of ROTEM are scarcely commented in our manuscript. Nevertheless, we have added FIBTEM-ROTEM instead ROTEM, where appropriate,

If minimum running time is 5 – 10 mins in these POC-tests, correction of low fibrinogen levels can be accomplished fast. Please compare timing until correction of trauma-induced coagulopathy in Schoechl et al. Crit Care 2010 and add this reference.

Response
We now mention the advantage the ROTEM compared to conventional laboratory tests, and have added the reference of Schöchl Crit Care 2010 (reference 24). Please, see discussion section, page 13, of the revised version.

p. 14:
What were the average times until transfusions and FBNc were on board of the bleeding patients? This quality indicators should be added in the paper.

Response
Ninety - five percent of patients received the first package of blood components within 40 minutes from the activation of massive transfusion protocol. In contrast, the average time for administration of FBNc was longer and variable (3 [2, 6] hours). This was because our protocol (figure 1) dictates that FBNc is to be administered always after transfusing the first package of massive transfusion. In a recent study dealing with bleeding trauma, the administration of coagulation factors was delayed 1 to 24 hours from the onset of bleeding in nearly one-half of patients (Schöchl Crit Care 2010)

We acknowledge that early treatment with coagulation factor concentrates might result in improved treatment of coagulopathy, and it could also avoid the side effects of plasma administration.
These considerations have been added into the results (page 9) and discussion sections (page 13)

For blood transfusions clear indications should be defined. Otherwise there is again a major limitation in this surrogate marker. Please add.

Response
We agree that clear indications for activating the MTP should be defined. Even though these were established in the first version of the manuscript (figure 1), we have rephrased this section for clarity (please, see methods, page 5). On the other hand, transfusion indications for non-massively bleeding patients are out of scope of this manuscript. We wish to underline that the hospital has a transfusion guideline, which may be consulted at any time on its intranet page. In addition, in Spain, the use of all transfusion alternatives are based on the recommendations issued at the Seville’s document (#reference 12). Please, see these considerations in methods section of the revised version of the manuscript (page 5).

If corrected late, efficacy of FBNc should not be different but of course FBNc is not “a magic bullet” that corrects thrombocytopenia plus hyperfibrinolysis plus lack in thrombin generation (INR was significantly higher in the FBNc group?!). It is required in several comprehensive algorithms that these pathomechanisms are corrected in parallel to hypofibrinogenaemia (hyperfibrinolysis needs to be stopped first) (compare e.g. Weber et al. Anesthesiology 2013). Accordingly, the authors need to check laboratory results of their patients also for the presence or absence of these other pathomechanisms. If they were present and not corrected alongside with FBNc administration then it is clearly not an inefficacy of FBNc but inefficacy of bleeding management. If these laboratory data cannot be delivered for such a categorization then at least this major limitation needs to be clearly outlined in the paper’s discussion.

Response
We agree. Fibrinogen concentrate is not a magic bullet that may be used in all the clinical settings whatever the patient presents massive bleeding. In addition, it should be prescribed in a timely timing and dosage. As established in the manuscript, we prescribed FBNc according to a comprehensive scheme (please, see figure 1), whenever the first package of massive transfusion had failed in stopping the bleeding and correcting the coagulopathy. We administered a median dosage of 2 g (25 mg/kg) that, even being low, is within the range recommended by the European guidelines. (this is recognized in page 13) Our trauma and surgical patients are treated according with the Spanish Consensus Statement on alternatives to allogeneic blood transfusion: the 2013 update of the "Seville Document" (Leal-Noval et al, Blood Transfusion 2013, reference 12), based on the concept of Blood patient management. In accordance with these guidelines, all trauma patients receive 3 g of tranexamic acid shortly upon admission to hospital, and always before FBNc administration. Tranexamic acid is also prophylactically administered in major surgeries, including cardiac and liver transplantation.
We deem important to highlight that whenever the massive transfusion protocol [figure 1] is activated, patients receive plasma, platelets and pRBC, as indicated in guidelines, in a fast and comprehensive manner. However, when reviewing our MTP, we realized that indications for administering FBNc should be modified, since its late and insufficient administration did not result in improved clinical outcomes. The new version of the manuscript recognizes that our results neither suggest nor support the lack of efficacy of FBNc but rather the probable inefficacy of an inappropriate use. This is clearly established in our conclusions, at the end of discussion section.

We have revised our data, which are correct. Due to the delayed administration of FBNc, it seems reasonable that the FBNc group had discretely more abnormal clotting tests. Please, note that platelets counts, INR and aPTT differed slightly between groups (e.g., INR 1.7 vs. 1.4; a difference which probably is barely important from a clinical point of view).

*Probably it is not earlier administration and higher doses of FBNc that would stop bleeding but embedding FBNc into a comprehensive goal-directed POC-guided algorithm as suggested e.g. by the ESA guidelines.*

Response

This is recognized in the new version of our manuscript.

**Key messages:**

*Low doses could be replaced by “fixed doses of 2 g”.*

Response

We disagree at this point. A median dosage of 2 [2, 4] is not a fixed dosage.

**Reviewer 3**

*Please note that the manuscript has been rewritten completely in line with the suggestions of the reviewers and the editor.*

This single centre retrospective cohort study design inherently has methodological limitations. In this study it is particularly evident that a number of factors has not been controlled for. The issue of selection bias, the timing of FBNc and transfusions, and the dosage FBNc are probably the most prominent.

Response

We agree that the study has methodological limitations inherent to its retrospective design. We have tried to overcome these limitations by performing two multivariate regression analyses and a matching process. We acknowledge these limitations in the revised version of the manuscript; please, see the discussion section (page 14).

As stated in table 1, the fibrinogen concentration, INR and BE are significantly lower in the FBNc group. These patients thus appear to be significantly worse off.
than the group they are compared to. The fact that the FBNc group receives significantly more blood units is therefore not surprising.

Response

Except for fibrinogen concentrations (1.3 g/l vs. 2.0 g/l; FBNc group vs. comparator group), the remaining analytical data did not greatly differ between groups [-4.3 vs. -4.0 for BE; 1.7 vs. 1.4 for INR, and 93 x 10^9 vs. 100 x 10^9 for platelets counts], and these differences probably had scarce clinical significance. We agree that patients from the FBNc group were sicker than those from the comparator group and, as a consequence, they received more blood components. Because of this, we re-analyzed transfusion data after a patients’ pair matching based on SOFA scores. After performing this analysis, no differences with respect to blood transfusion were observed.

I struggle to see how the within-group comparison (table 2) can be justified in this setting, other than to observe that laboratory parameters does not change as expected. The fact that the fibrinogen concentration does not change significantly after administration, clearly demonstrates that the dosage is too small. (Note: the numbers in the text does not match the ones in table 2). The primary outcome (transfusion requirements) in the within-group comparison is in my opinion hardly of any value, especially as long as no time factor is indicated over which the transfusions are administered. Moreover, as FBNc is administered relatively late, surgical haemostasis is more likely to be achieved (and possibly surgery discontinued) soon after the drug has been given. And thus, the number of transfusions after administration of FBNc is lower.

Response

We have reviewed our database and added new data, including timing of administration for blood transfusion and FBNc. (please, see results section, page 9).
On the other hand, a significant change in fibrinogen levels and FIBTEM measurements might not be detected, even 24 hours after the administration of high doses of plasma or fibrinogen. In trauma patients, despite the administration of high doses of FBNc (median 6 g), the mean fibrinogen plasma level was not within range until 24 hours after its administration (Schöchl H Crit Care 2010). Massive plasma transfusion does not improve ROTEM values after 1 day from its administration (Khan S, J Trauma 2014;76:561). So, fibrinogen may reach an optimized range (≥ 1.5 - 2 g/l) lately.

I find the statistics between-groups comparison section somewhat confusing. The ANOVA analysis is of interest as it may to some extent compensate for the selection bias, but I believe the intention of performing these analyses needs to be more clearly justified in the text.
The matched between-groups analyses are in principle of some interest. The number of included subjects is however relatively low, and baseline fibrinogen still significantly higher in the comparison group.

Response

We have explained clearer and more comprehensively the statistic analyses in
the revised version of the manuscript (please, see new figures 2 and 3, and statistical analysis). Patients were pair-matched mainly based on SOFA scores. Notwithstanding, a difference between baseline fibrinogen levels was still found (1.7 g/l vs. 2.0 g/l, for FBNc group and comparator group, respectively; p=0.04), but it probably was of little clinical relevance (3 tenths).

The discussion section adequately pinpoints many of the limitations of this study, but the outline may be somewhat improved. The conclusion is founded in the data presented.

In summary, this study raises an interesting question, however methodological shortcomings and the presentation of the analyses limits its value. Suggestions for improvement would be to narrow down the results section, focusing on the most important analyses and data points. The rationale for performing the analyses should be made clear. References to tables, explanation of abbreviations and consistency of numbers between tables and text should be revised.

Response

We have taken in account all the reviewer’s suggestions. Results section has been narrowed down, and statistical analyses clearer explained.

Figure 1 is hardly readable in my version of the manuscript. This is probably due to formatting.

Response

We now enclose figure 1 in a different format and higher resolution.

The manuscript also needs considerable language improvements. This is not commented in further detail in this review.

Response

We have tried to improve the manuscript language with the help of a native English speaker.