Author’s response to reviews

Title: Could enteral nutrition improve outcome of patients with haematological malignancies undergoing allogeneic haematopoietic stem-cell transplantation? (NEPHA study): study protocol for a randomized controlled trial

Authors:

Richard RL Lemal (rlemal@chu-clermontferrand.fr)
Aurélie AC Cabrespine (acabrespine@chu-clermontferrand.fr)
Bruno BP Pereira (bpereira@chu-clermontferrand.fr)
Cécile CC Combal (ccombal@chu-clermontferrand.fr)
Aurélie AR Ravinet (aravinet@chu-clermontferrand.fr)
Eric EH Hermet (ehermet@chu-clermontferrand.fr)
Jacques-Olivier JOB Bay (jobay@chu-clermontferrand.fr)
Corinne CB Bouteloup (cbouteloup@chu-clermontferrand.fr)

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Author’s response to reviews: see over
Dear Editor,

We are submitting today our revised manuscript entitled "Could enteral nutrition improve outcome of patients with haematological malignancies undergoing allogeneic haematopoietic stem-cell transplantation? (NEPHA study): study protocol for a randomized controlled trial" for publication in Trials.

This manuscript was initially submitted as a Study Protocol, and describes the trial we designed in order to randomly compare enteral and parenteral nutrition support in allogeneic haematopoietic stem cell transplantation (allo-HSCT).

We received reviewers comment on January, 21st 2015 and addressed a first revision in February, the 16th.

The handling editor has requested (February, the 26th) that we revisit the report from reviewer 1, as he did not feel that we addressed his original comments in our revisions.

Please find below our point-by-point answer to the concerns. All comments have been answered, one-by-one. And all required modifications have been added in the revised manuscript, appearing yellow highlighted.

We confirm that the revised manuscript and the present covering letter have been read and approved by all named authors.

Thank you in advance for your kind consideration of this paper.

Best regards,

Dr Richard Lemal, on behalf of co-authors.
Editorial requests

"1. Please ensure the title conforms to journal style for study protocol articles. The title should follow the format ?________: study protocol for a randomized controlled trial.?"

2. Please replace the Conclusion in your Abstract with a Discussion.

3. Please include the date of registration with the trial registration number at the end of the Abstract.

4. Please include the full names of all ethical bodies that approved your study in the various centres involved, along with the reference numbers provided, in the Methods section. If you do not wish to list them all in the Methods section, please include the list as an additional file and refer to this in the Methods section.

5. Please include your funding information in an Acknowledgements section at the end of the manuscript, before the reference list”.

All editorial requests have been taken into account: modifications appear yellow highlighted in the manuscript. We would like to be more precise about the request 4: in France, there is no need for multiple ethical bodies approval when several centres are involved. The local ethical body "CPP Sud Est VI" approved NEPHA trial and this agreement is valid for all the sixteen reported french centres.

Reviewer 1

I found this to be a weak protocol. Too many data is taken on too few patients; the statistical plan is vague and ambiguous.

We take note of reviewer's advice on this trial's quality. Nevertheless, we would like to address the following comments.

NEPHA trial received a grant from French Ministry of Health, after a strict and highly selective process. During the selection process, NEPHA has been evaluated and rated by international experts in the field of oncology, haematology and nutrition. Moreover, some of the reviewer's comments (notably regarding the high number of data and endpoints) concern data and endpoints which have specifically been requested by international experts during the selection process.

Finally, NEPHA trial is conducted with institutional support from the french society of clinical nutrition and metabolism (SFNEP) and from the French Society for hematopoietic stem cells transplantation and cellular therapy (SFGMTC). NEPHA trial has been approved unreservedly by these societies.
1. Too many data. If you look at table 2, there are 27 endpoints (one of which has several domains) repeated up to 10 times. There are therefore nearly as many endpoints as patients.

Allogeneic haematopoietic stem-cell transplantation (allo-HSCT) is a heavy and complex procedure: close attention must be paid to subtle details at time of results analysis.

In NEPHA trial, we clearly defined one main endpoint (day-100 survival) which is clearly enounced.

Beside this main objective, it is usual to take into account all the main issues which can impact on morbidity and mortality in allo-HSCT: these six secondary endpoints are clearly enounced in the "haematologic evolution", namely: GVHD (incidence and severity), secondary toxicities (namely, infections and mucositis), and haematopoietic reconstitution. In practice, it is impossible to correctly assess such complex and dynamic issues with a single punctual evaluation. For example, a correct analysis of infectious events is not possible without an iterative multiparametric evaluation of infectious clinical signs, biological inflammation parameters, bacteriological documentation and antibiotic, antifongic and/or antiviral use.

Regarding nutrition issues, four secondary endpoints are clearly enounced to be evaluated, namely: nutritional status, functional status, duration of nutritional support and tolerance of nutrition procedure. Once again, it is impossible to correctly assess such complex and dynamic issues with punctual and monoparametric evaluation.

Such iterative and multiparametric evaluations are usually done and reported in all allo-HSCT trials. The three retrospective studies published in this specific "enteral nutrition / allo-HSCT" field (quoted in reference section, 23 - 25) used the same methodology and criteria.

Most analyses of secondary outcome parameters will be exploratory and should have been underpowered. Feise (2002) consider that, even if a study needs to be statistically analyzed at all, attention must be paid to focus not only on statistical significance (adjusted or not), but also on the quality of the research within the study and the magnitude of improvement. (Rothman, KJ.; Greenland, S. Modern Epidemiology. 2nd ed. Philadelphia: Lippincott Williams &Wilkins; 1998; Ottenbacher KJ (1998) Quantitative evaluation of multiplicity in epidemiology and public health research. Am J Epidemiol 147: 615–619; RJ Feise. Do multiple outcome measures require p-value adjustment? BMC Medical Research Methodology 2002, 2:8)

We added appropriate comments in the revised manuscript, in "Evaluation of outcomes" section.

2. Too few patients. The authors have set the event rates as 17% and 5%, suggesting that over 70% of the deaths in this patient group are due to correctable nutritional problems. That strikes me as extremely implausible.

Rare data available in retrospective studies in this "enteral nutrition / allo-HSCT" field (quoted in reference section, 23 - 25) mentioned event rates for overall survival at day-100 with myeloablative conditioning regimens respectively at 33% in PN group vs 8 % in EN group, suggesting that 75% of early deaths could be avoided with EN.
Even if these results can appear "implausible" considering solely "correctable nutritional problems", we would like to address reviewers some remarks:

- as mentioned in the Background section, several studies showed that malnutrition is an independent negative prognostic factor for the survival of children and adults affected by malignant haematological diseases and treated by allo-HSCT. Thus, nutritional problems remain a major, central and under-estimated actor to improve in allo-HSCT

- recent published data reported on the major impact of dysbiosis on survival after allo-HSCT; microbiota being suspected to be involved in immunological process regulating GVHD pathophysiology and in immunological regulation of infectious events. Enteral nutrition is known to deeply and directly impact on microbiota composition. So, even if these issues are not directly explored by our study, data suggesting a mechanistic explanation for EN expected superiority exist.

Thus, sample size has been statistically estimated as usually recommended, regarding all the recently published data available precisely in this restricted investigation field. Even if the expected results may seem counter-intuitive, it could be partially explained by recent scientific data suggesting that route of nutrition administration could impact on more than nutritional status.

Lastly, we would like to put this study back in its proper context, namely allo-HSCT trials context: allo-HSCT with myeloablative conditioning regimens are not a frequent setting and it remains nowadays uneasy to include lots of such patients in prospective trials, as evidenced by the lack of large prospective trials in this field.

We added appropriate comments in the revised manuscript, in "Discussion" section.

3. Statistical plan too vague. The ideal for a statistical analysis plan is that two independent statisticians receiving the trial data set could follow the plan and come up with virtually identical sets of results. This cannot be the case if the protocol states that although one test is planned, another might be used “if necessary” and that a third approach “could be envisaged”.

We agree that "statistical considerations" section presented in our previous version could be considered as “too vague” regarding some points. According to our institute’s experience in Trials Journal (8 publications to date), we aimed to fulfill usual editorial and reviewers requests, reporting enough details in statistical considerations section, without being as fairly complex as could be a statistical plan.

We strove to always enounce the more appropriate statistical analyses, in accordance with their assumptions and validity conditions.

We agree that some terms are inappropriate: for instance “if necessary” or “could be envisaged”. We adjusted these terms in the revised manuscript.

In some cases (e.g. time to event) two separate analyses are planned (log rank and Cox regression).

Two separate analyses are not planned systematically. We aimed to distinguish univariate and multivariate analyses, as specified in the revised manuscript.
The plans for many endpoints are extremely vague (the protocol states only that they will be analyzed using mixed models). The protocol should explicitly state a single analysis planned for each and every hypothesis with a justification for each.

We thank the reviewer for his helpful comment which allows us to give more details concerning the longitudinal analysis.

All criteria planned to be analyzed using mixed models are frequently used to assess nutritional status: weight, body-mass index and brachial circumference are morphometric measures; muscle strength measurement reflects functional status; albumin and transthyretin are biological parameters for nutritional evaluation; C-reactive protein is an inflammatory marker, used to interpret albumin and transthyretin, which can be altered in inflammatory states.

Only rare, incomplete and retrospective data are available in the literature (ref 23-25) in this specific field. Even if it remains uneasy to anticipate the results, we can assume, according to our previous unpublished experience, that morphometric measures and biological markers will show a better nutritional status in "enteral nutrition" group. In correlation with these results, functional status should be better in the EN group.

The randomized nutrition procedure is planned to be administered, on average, during 21 to 28 days: thus, we assume that improvement of nutritional status in EN group should be more relevant within the first 2 to 3 months after allo-HSCT, thereafter the nutritional status should not be significantly different between the two groups.

Careful considerations for these questions have been added in the revised version of manuscript (in "Nutritional outcomes" section).

4. The statistical approaches are questionable in many cases. Some endpoints to be assessed by t-test (e.g. number of days of re-hospitalization) are Poisson in nature, and should be tested by Poisson or negative binomial.

We understand the relevant comment from the reviewer, but we would like to highlight that it seems difficult to propose an appropriate statistical approach according to a priori unknown statistical distribution. Even if it seems reasonable to assume that certain parameters such as number of days of re-hospitalization are Poisson in nature, we propose therefore to specify "according to statistical distribution such as Poisson or negative binomial".

This has been added in the revised manuscript.

Some endpoints, such as survival, are described in terms of Cox or log rank even though the protocol states that these are binary endpoints at one year.

As clearly mentioned in the initial manuscript, early mortality (at day 100 [D100]) and overall survival at one year will be analyzed distinctively. In these settings, early mortality will be analyzed as a binary parameter, with a Cox test and overall survival at one year will be analyzed as censored data, with a log rank test.
We would like to clarify that early mortality at D100 is a strong marker of early allo-HSCT toxicity, classically used in all allo-HSCT trials. This endpoint is always considered as a binary parameter, without right-censored data.

Overall survival and progression-free survival at one year will be considered as censored data. Then, we specify that these censored data will be analyzed by specific appropriate methods (Kaplan-Meier, etc.).

**Reviewer 2**

1. **Authors only mention center as a stratification factor. This is arguably the most relevant, but factors related to disease severity as well as conditioning and prophylactic regimens are probably very important as determinants of the primary endpoint, overall survival at 1 year. If these or other stratification factors are not contemplated, authors should at least explain why and discuss the potential resulting imbalance in prognostic factors for 1-year survival between arms.**

NEPHA trial’s randomisation procedure underlie on a stratification by center, which aims to limit the effect of some local practices which could interact with the main or secondary endpoints. These local practices, for which no consensual nor unambiguous international recommendations exist, can be linked for instance to:

- the central venous access used (with or without tunnelling, implantable device...)
- different approaches regarding the time to ICU transfer (as soon as an "organ instability" can be detected in some centers vs at "septic shock" in others...)

Regarding the nature of conditioning regimens, we chose to limit the inclusions to the myeloablative conditioning regimens (as enounced in Inclusion Criteria). This choice was made in order to limit the heterogeneity of conditioning regimens used and its impact on mortality at D-100 and on overall survival at 1 year. In order to include only patients with close intensity regimens, we centrally check conditioning regimens intensity for each inclusion request and before randomisation.

Finally, we can assume that drugs administered as prophylactic regimens towards GVHD will not be imbalanced between the two groups, for the following reasons:

- we chose to exclude patients with HLA-compatibility < 10/10, in order to include only patients with less risk of GVHD. This should lead to limited prescription of unusual prophylactic immunosuppressive drugs.
- we chose to exclude participation of patients who can receive an experimental or innovative prophylactic regimens or graft. This should lead to focus on patients receiving usual prophylactic regimens, according to center habits.

We explained and discussed these choices in the "Randomisation" section of the manuscript.

2. **Authors should explain why they believe there will not be right-censored data. Is it because the analysis will be conducted after all patients have been observed to Day 100?**

We would like to clarify that early mortality at D100 is a strong marker of early allo-HSCT toxicity, classically used in all allo-HSCT trials. This endpoint is always considered as a

Mortality at D100 estimation relies on a binary parameter, which is always available for all patients, by close monitoring within a short period of time.

In order to improve our results reliability, we proposed to complete this evaluation with an evaluation of overall and progression-free survivals at one year, which will be considered as censored data and analyzed with appropriate tests.

3. In the very last sentence of the Discussion, authors imply that an element of adaptation is contemplated. However, this has not reportedly been taken into account in the study design. Authors should explain how this issue would be dealt with formally should the need arise for sample-size increase.

Actually, this manuscript was drawn up a few months after trial design and recruitment beginning. As a consequence, and on the basis of recent published data regarding the recent improvements described in the manuscript, we expect that toxicity could be lower in the control arm (PN), leading to an insufficient statistical power.

We just aimed to anticipate this risk as a possibility in the Discussion section of the manuscript, without contemplating such adaptation. The last sentence, which clearly could be misleading, has been removed from the manuscript.