Author's response to reviews

Title: Cytokine serum levels during post-transplant adverse events in 61 pediatric patients after hematopoietic stem cell transplantation

Authors:

Michaela Döring (michaela.doering@med.uni-tuebingen.de)
Karin M Cabanillas Stanchi (karin.rohrer@med.uni-tuebingen.de)
Markus Mezger (markus.mezger@med.uni-tuebingen.de)
Annika Erbacher (annika.erbacher@med.uni-tuebingen.de)
Judith Feucht (judith.feucht@med.uni-tuebingen.de)
Matthias Pfeiffer (matthias.pfeiffer@med.uni-tuebingen.de)
Peter Lang (peter.lang@med.uni-tuebingen.de)
Rupert Handgretinger (rupert.handgretinger@med.uni-tuebingen.de)
Ingo Müller (i.mueller@uke.de)

Version: 2 Date: 11 June 2015

Author's response to reviews: see over
Re: Cytokine serum levels during post-transplant adverse events in 61 pediatric patients after hematopoietic stem cell transplantation

Dear Dr. Dafne Solera,

Please find enclosed the revised manuscript “Cytokine serum levels during post-transplant adverse events in pediatric patients after hematopoietic stem cell transplantation” by Michaela Döring et al. for re-submission to *BMC Cancer* as a full length article.

We would like to address each of the reviewer’s proposals point by point as follows:

**Additional Editorial Request:**

1. Ethics - Please revise the Methods section of your manuscript to include the name of the ethics committee that waived approval for your study. We recommend the following format: "Approval for this study was waived by the ethics committee of [xxx] Hospital [or University]."

A: We have added the ethical statement and additionally uploaded the Ethical Approval Form of the Institutional Ethics Committee.
“This analysis was conducted in accordance with the Declaration of Helsinki and performed under the waiver for retrospective anonymized studies and in accordance with the Independent Ethics Committee (IEC) of the Eberhard-Karls-University Tübingen.”

2. Line numbering - Please include continuous line numbering in your revised manuscript to facilitate re-review.

a: Line numbering has been added.

Reviewer #1:
Reviewer's report
Title:Cytokine serum levels during post-transplant adverse events in 61 pediatric patients after hematopoietic stem cell transplantation: a retrospective analysis
Version:1 Date:13 April 2015
Reviewer:Grant Trobridge
Reviewer's report:

Major Compulsory Revisions
1) The first sentence of the Discussion states that "The objective of this study was the timely identification of major post-transplant adverse events allowing an early start of adequate treatment in pediatric patients...". However this was a retrospective study. They did not test whether an approach could identify adverse events. As the authors point out later in the manuscript additional prospective studies will be needed to determine if the parameters defined here can accurately predict adverse events. This should be made clear here and in the Conclusions.

A: We agree with the reviewer and revised the according sections.

Results: "This retrospective study analyzed the role of the cytokines IL-1β, sIL-2R, IL-6, IL-8, IL-10 and TNF-α as potential markers for major post-transplant adverse events including VOD, skin and intestinal GvHD, sepsis as well as bacterial, viral and fungal infections in 61 pediatric patients."

Discussion: “The primary objective of this retrospective study was to analyze whether early identification of major post-transplant adverse events in pediatric patients with hematono-cological malignancies and non-malignancies after HSCT is possible through the examination of cytokine levels. Early identification of these post-transplant adverse events is required for timely and adequate treatment and thus has decisive impact on patient outcome."

Conclusions: “The presented retrospective study shows that the analysis of cytokines enables differentiation of major post-transplant complications. A significant increase in cytokine levels of IL-6, IL-8, and TNF-α announces the beginning of a VOD. For suspected cases of intestinal GvHD ≥ grade II, a significant increase of cytokines IL-6, IL-10, sIL-2R and TNF-α may serve as an early identification marker. A significant increase of IL-6 alone was associated with ADV-viremia and significant increases of IL-6 and IL-8 with bacteremia. Separate from this, a sepsis was characterized by significant increases of IL-6, IL-8 and sIL-2R. Analysis of the cytokines allowed differentiation of post-transplant adverse events with similar clinical symptoms (for example intestinal GvHD and diarrhea due to viral infection, or VOD and liver GvHD). However, studies with larger patient cohorts and a prospective setting will be performed to validate these conclusions in order to use characteristic cytokine patterns to identify post-transplant adverse events as early as the onset of fever with unknown origin or other initial clinical symptoms, and thus facilitate a correct treatment approach.”

Minor essential revisions

2) The beginning of the Results section should have 1 to two sentences as an overview of the study setting the stage for approach and rationale.

A: We agree with the reviewer and have revised the section.

Discussion: “The primary objective of this retrospective study was to analyze whether early identification of major post-transplant adverse events in pediatric patients with hematono-cological malignancies and non-malignancies after HSCT is possible through the examination of cytokine levels. Early identification of these post-transplant adverse events is required for timely and adequate treatment and thus has decisive impact on patient outcome."

3) The abstract does not have abbreviations defines and sIL2 is used in place of sIL-2R.

A: Corrections have been made, accordingly.

Reviewer #2:
Reviewer's report
Title:Cytokine serum levels during post-transplant adverse events in 61 pediatric patients after hematopoietic stem cell transplantation: a retrospective analysis
Version:1 Date:8 May 2015
Reviewer:Sinisa Dovat
Reviewer's report:

Major Compulsory Revisions:
1) In order to better understand the association of elevated cytokines with a particular adverse event post transplant, it is essential to clarify whether there is a difference in cytokine levels due to a particular conditioning regimen and/or due to the type of transplant performed. Specifically, the levels of cytokines in patients who have received total body irradiation (TBI) as part of conditioning should be compared to the cytokine levels in patients who did not receive TBI (in a table).

A: We agree with the reviewer that comparison of the different transplantation strategies and irradiation is interesting data and helps the reader to evaluate our findings. We have re-analyzed the data and added the according data in a separate section in the manuscript.

Results: "Cytokines and stem cell transplantation. The analysis of the cytokine level in the different types of stem cell transplantation and conditioning regimen occurred at median on day +2 (range +1 to +4) after HSCT. The comparison of patients with versus without TBI did not reveal any difference in any of the cytokines analyzed (IL-1β: P=1.0; sIL-2R: P=0.228; IL-6: P=0.912; IL-8: P=0.645; IL-10: P=0.868; TNF-α: P=0.433)."

2) Similarly, cytokine levels in patients who received stem cells from matched unrelated donors (MUD), should be compared to cytokine levels in other patients.

A: We have added the information likewise.

Results: "As well, comparison of cytokine levels between MUD and MMFD showed no significant difference (IL-1β: P=0.123; sIL-2R: P=0.588; IL-6: P=0.494; IL-8: P=0.695; IL-10: P=0.793; TNF-α: P=0.426). In contrast to this, the comparison of MUD and MFD showed significant differences for cytokines IL-1β (mean 0.134±0.058 pg/ml versus 0.624±0.184 pg/ml, respectively; P=0.0019), sIL-2R (mean 1431±1076 U/ml versus 550±165 U/ml, respectively; P=0.0185) and IL-8 (mean 46.3±37.6 pg/ml versus 16.0±11.7 pg/ml, respectively; P=0.023). Levels of IL-6 (P=0.067), IL-10 (P=0.221) and TNF-α were not significantly different in these two groups."

3) How many patients that were diagnosed with VOD had at the same time GVHD Grade III or IV?

A: None of the patients with VOD had GvHD ≥ grade III at the same time. The according information has been added to the manuscript text.

Results: "None of the 5 pediatric patients with VOD had a GvHD grade III or IV or a sepsis simultaneously."

4) How many patients diagnosed with VOD had at the same time documented sepsis?

A: None of the patients with VOD experienced a sepsis at the same time. The according information has been added to the manuscript text.

Results: "None of the 5 pediatric patients with VOD had a GvHD grade III or IV or a sepsis simultaneously."

5) How many patients diagnosed with VOD have documented VOD by liver biopsy?

A: VOD was diagnosed according to the internationally accepted and used Seattle or Baltimore Clinical Criteria as stated in the manuscript text [1]. All of the 5 patients with VOD were diagnosed without liver biopsy. According information has been added to the material and methods section.

M&M: "Diagnosis of VOD was performed according to the Seattle or Baltimore clinical criteria. No liver biopsy or analysis of plasminogen activator inhibitor-1 (PAI-1) level was performed in patients diagnosed with VOD.

6) In patients diagnosed with VOD, who did not undergo liver biopsy, how many of them had elevated levels of PAI-1?

A: The plasminogen activator inhibitor-1 (PAI-1) level was not checked for any of the patients with VOD.

According information has been added to the material and methods section.

M&M: “Diagnosis of VOD was made according to the Seattle or Baltimore clinical criteria. No liver biopsy or analysis of plasminogen activator inhibitor-1 (PAI-1) level was performed in patients diagnosed with VOD.

7) Discussion should be re-written and the emphasis should be placed on mechanistic rationale for the observed results, along with future research directions regarding the role of cytokines in adverse effects of stem cell transplants. The readers of BMC Cancer would benefit from a discussion that consists of more forward thinking regarding diagnostic and therapeutic options in pediatric patients with stem cell transplants.

A: We agree with the reviewer and have revised the discussion section accordingly (See pages 14-16 in the manuscript text; sections Discussion and Conclusions).
We would like to thank the reviewers for the helpful comments and criticism and would be very happy if you consider the manuscript suitable for publication in *BMC Cancer*.

If you have any questions or suggestions, please do not hesitate to contact me.

Yours sincerely,

Michaela Döring, MD

References