Peer Review File

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Reviewer A

This MS summarizes results of a structured literature review (meta-analysis) focusing on the effects of glutamine supplementation on outcome (primarily in-hospital mortality) variables. The general design of the study follows internationally accepted methodology.

Major concerns:

Comment 1: The scientific rationale behind the study is generally questionable. Glutamine is a nutrient, not a drug! So, any evaluation of clinical studies with respect to potential glutamine effects must (!) consider the general nutrition therapy used including energy, essential nutrients like amino acids, fatty acids micronutrients etc. Glutamine supplementation alone without an adequate nutritional support covering the requirements of patients cannot help! That the consideration of glutamine does not work is simply shown by the Heyland study (ref. 7): as broadly discussed in the scientific community, most of the patients received too less energy and too less nitrogen (protein, amino acids). Obviously, the present evaluation did not report nutrition intake in the various studies; it is, however, to expect that these intakes (energy, nitrogen etc.) broadly varied. This factor alone prohibits comparably evaluation of effects of glutamine supplementation. Moreover, different therapeutic concepts (eg, surgical strategies, medication) may influence glutamine effects.

The use of glutamine is based on the original idea that glutamine is a conditionally independent nutrient and should be part of an adequate nutritional support (like other essential nutrients!). If glutamine is not given, there is a high risk that the sick body cannot optimally react on medical therapy. The literature of clinical studies does not know trials evaluating whether the inadequate supply of an essential nutrient will increase patient mortality! So, why this has to be shown for glutamine? Please, explain. Official glutamine recommendations (ESPEN, ASPEN etc.) only focus on mortality and, probably, morbidity not considering overall nutrition concepts. This is, indeed, a concept which is used for drugs.

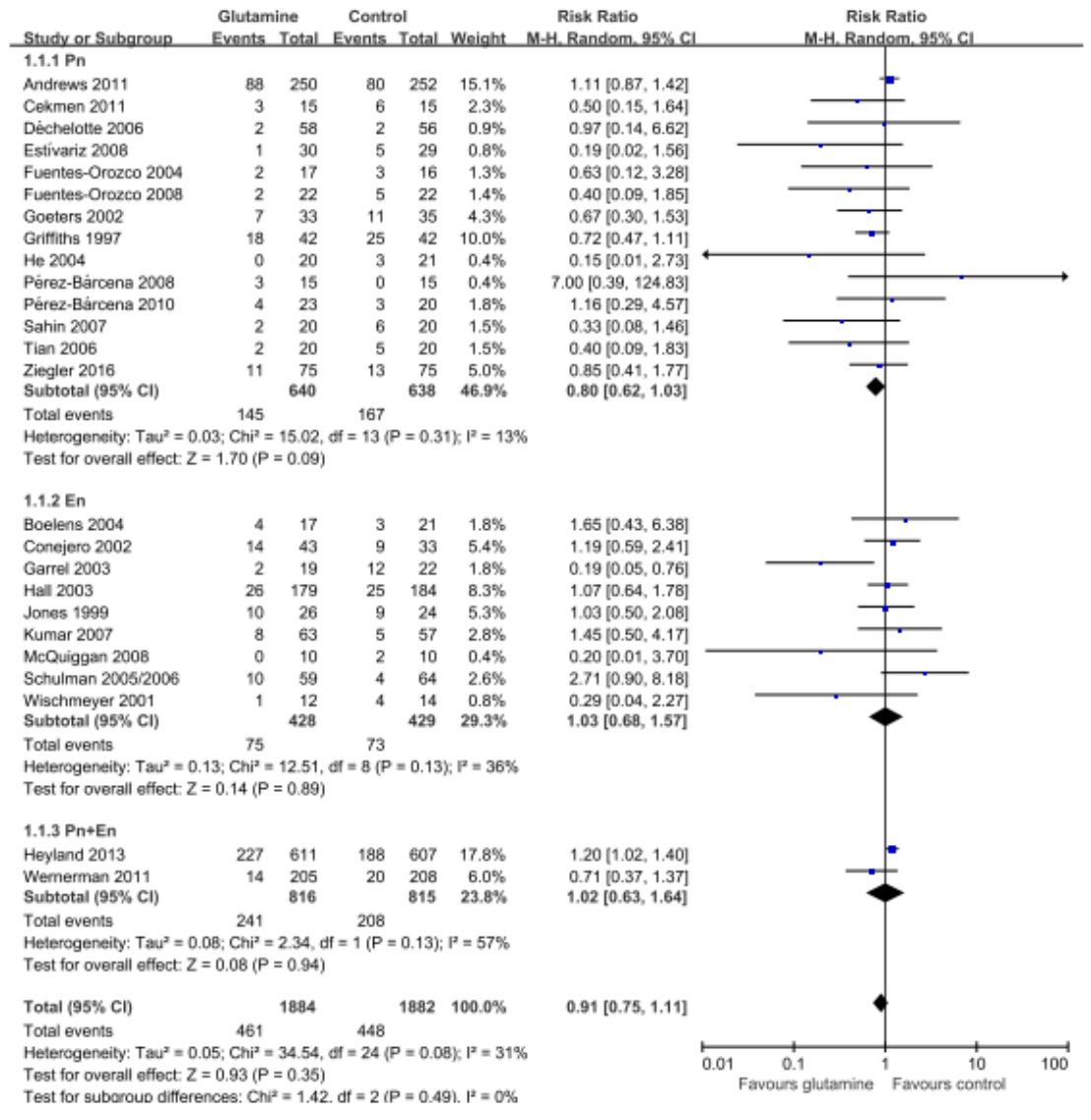
Reply 1: Thank you very much for your comment and we agree with it. Glutamine is an essential nutrition indeed ,but it is also a key for maintaining intestinal barrier integrity and function especially in critically ill patients. A study showed that bacterial translocation and gut-origin sepsis may be involved in the pathogenesis of systemic infectious complications and multiple organ deficiency syndromes (Deitch EA. Gut-origin sepsis: evolution of a concept. Surgeon. 2012, 10(6): 350-6. doi:

10.1016/j.surge.2012.03.003.). So we focused on the effect of glutamine supplementation (enteral or parenteral) on mortality in critically ill patients and also investigate the impact of glutamine on the other outcomes .

Changes in the text: We added some data to explain glutamine may have an effect on maintaining intestinal barrier integrity and function against sepsis or MODS(see Page 5, Line 13-15).

Comment 2: Another crucial point is the idea not to separate enteral and parenteral glutamine supplementation in this evaluation. It has been shown in various clinical studies that the way of nutrient supplementation influences the time course and the effectiveness of nutrition therapy. Moreover, depending on the kind of enteral products used to nourish the patients, these industrial products already provide glutamine either bound in proteins, peptides or as free glutamine. So, the total glutamine intake must be calculated considering all of these data. In addition, it should be reported in which form glutamine has been given (in free form or as dipeptide).

Reply 2: Thank you very much for your comment and we agree with it. Firstly, we attempted to separate nutritional modes into enteral, parenteral and a combination of them. The results are as follows. However, in three subgroups, there was no significant difference in hospital mortality between GLN group and control group (Pn: RR 0.80, 95%CI, 0.62 to 1.03, P=0.09; En: RR 1.03, 95%CI, 0.68 to 1.57, P=0.89; Pn+En: RR 1.02, 95%CI, 0.63 to 1.64, P=0.94), although this was not statistically significant after considering the test for interaction P values (interaction P=0.35). We deleted the results because of no significant difference and the limition of article length.



Secondly, the supplement of glutamine was explained into two ways: the total glutamine or the rate calculated by per kilogram(kg) per day(d). The glutamine were either bound in proteins, peptides or as free glutamine just as you mentioned in your comments ,so it was difficult for us to completely separate them indeed. The supplement of glutamine in some studies calculated as dose in proteins (Boelens 2004), in some studies were free glutamine (McQuiggan 2008). To be in line with the guideline announced by the Society of Critical Care Medicine and the American Society for Parenteral and Enteral Nutrition, we chose the rate calculated by per kilogram(kg) per day(d).

In our studies, some patients were given L-alanyl-L-Glutamine and others were given Glutamine. We didn't choose it as a subgroup factor this time. Thank you for your insightful suggestion.

Changes in the text: We have modified our text as advised (see Page11, Line8-15; Page13, Line1-9).

Comment 3: The last point is the dosage. One Evaluation arm should check whether the dosage is influencing metabolic/clinical effects.

Reply 3: We agree with your comment. We have already analysized whether the dosage affect metabolic/clinical effects. Subgroup meta-analyses were conducted to estimate the effect of glutamine supplementation on specific dosages (above 0.5 g/kg/day, between 0.3 g/kg/day and 0.5 g/kg/day, below 0.3 g/kg/day).

Changes in the text: None.

Minor comments

Comment 1: Since 1990 (ref. 1), various reviews and comments have been published illuminating the role of glutamine in nutrition therapy. Please, add some new references. It is also mandatory to give some background why glutamine is not regular part of parenteral solutions. In addition, it should be noted that when enteral proteins as part of ready-to-use products are given, a considerable amount of glutamine is already included.

Reply 1: Thank you for your comment and we agree with it. We have added some new references in our manuscript (ref. 2-5).Conventional nutritional products for parenteral use do not contain glutamine due to glutamine instability in aqueous solution. All available enteral products for ICU nutrition contain glutamine, being a natural constituent of proteins, usually 7-8 % of the amino acid content. Plasma glutamine concentration may be decreased during intense immune cell activity in patients with critical disease conditions. In a wide range of catabolic/hypercatabolic situations (e.g., ill/critically ill), glutamine supplementation might be recommended to improve the prognosis of these patients. One meta-analysis showed that parenteral Glutamine (0.3-0.5g/kg/day) as part of nutrition therapy reduced infectious complications, ICU LOS, hospital LOS and mechanical ventilation duration in critically ill patients(Stehle et al 2017).

Changes in the text: We added some data and references (see Page 5, Line 2-6 and Page 17, Line 1-7).

Comment 2: Within the last years, several meta analyses focusing on the effects of nutritive glutamine supplementation has been published (eg, Stehle et al 2017). Only few of them are cited. Please, complete the list.

Reply 2: Thank you for your suggestions. We find two meta-analyses (Stehle et al 2017, Pimentel 2020) focusing on glutamine therapy in critically ill patients and added some data in our manuscript.

Changes in the text: We added some data and references (see Page 18, Line 19-21).

Comment 3: Table 1 is not readable.

Reply 3: Thank you for your kind reminder. We have already changed the format from excel to word.

Changes in the text: We have modified our Table 1 and resubmitted it to the editor's E-mail.

Comment 4: Limitations. All of the major concerns (see above) should be integrated in this chapter.

Reply 4: Thank you for your comment and we agree with it. We added some sentences in limitations and we are looking forward to your further comments.

Changes in the text: We have modified our text as advised (see Page 19, Line 1-4).

Comment 5: Conclusions. Again, following the limitations of the design, general conclusions on glutamine effects are not motivated.

Reply 5: Thank you for your suggestions. We emphasized that following the limitations of the design, general conclusions on glutamine effects are not motivated.

Changes in the text: We have modified our text as advised (see Page 19, Line 8-14).

Reviewer B

In my opinion there are a few aspects that you could have done differently in order to contribute to the knowledge regarding Glutamine supplementation. There are too many assumptions and generalizations with the current paper.

Comment 1: Dosage: When commenting on Glutamine effectiveness, you must differentiate between repletion versus supplementation. You cannot group studies that replete at doses of 0.2 g/kg in the same group as those that supplemented at 0.8 g/kg. You also must calculate and report the exact Glutamine dosages used in all the studies. It is not accepted to indicate values as > 0.5 g/kg. These were the exact studies that contributed the most weight in your meta-analysis and should be reported more accurately.

Reply 1: Thank you for your comment and we agree with it. To be in line with the guideline announced by the Society of Critical Care Medicine and the American Society for Parenteral and Enteral Nutrition, we chose the rate calculated by per kilogram(kg) per day(d). In both oral/enteral or parenteral nutrition, the typical glutamine daily administration (free and dipeptide forms) may vary from a fixed dose of 20-35 g/24 h to an adjusted dose of <1.0 g (usually 0.3 g-0.5 g) per kg of body weight (Stehle et al 2017). So one of our subgroup meta-analysis in this study was conducted by specific dosages, i.e., above 0.5 g/kg/day, between 0.3 g/kg/day and 0.5 g/kg/day, below 0.3 g/kg/day. The accurate dosage of glutamine was reported in

Table 1.

Changes in the text: None.

Comment 2: Combination therapy: You cannot also group studies that only added Glutamine to those that added Glutamine as part of a cocktail approach. How can you attribute the result to Glutamine if there were other nutrients that were also added? Please differentiate clearly between the different studies.

Reply 2: Thank you for your comment and we agree with it. All the studies we selected which were randomized controlled trails, and glutamine group was the experimental group. In these RCT studies, other factors have been balanced in each study.

Changes in the text: None.

Comment 3: Route: You indicated that some of the studies added the additional Glutamine via the enteral route, others via parenteral route and some were combination routes. Clearly you must study the effects of the various routes individually.

Reply 3: Thank you for your comment and we agree with it. We have added the data of different modes of glutamine supplement.

Changes in the text: We have modified our text as advised (see Page 15, Line 3-10).

Comment 4: Baseline glutamine status: Although not all studies indicate the plasma glutamine levels, you must at least acknowledge this as a serious flaw in your study. Please add a discussion about the effect of glutamine based on circulating plasma levels and link that to the outcomes assessed.

Reply 4: Thank you for your comment and we agree with it. We have added the data in discussion about the effect of glutamine based on circulating plasma levels and link that to the outcomes assessed.

Changes in the text: We added some data (see Page 15, Line 9-14).

Comment 5: You indicated that secondary outcomes like length of ICU stay, length of hospital stay, and nosocomial infections were included. You did not include results for any of these outcomes.

Reply 5: Thank you for your comment and we agree with it. We have added the data in results.

Changes in the text: We have modified our text as advised (see Page 11, Line 8-21; Page 12, Line 1-10, 19-21; Page 13 Line 1-21; Page 14 Line 1-10, 12-14; Page 15 Line 1-7).

Comment 6: In line 200 you note that “the compendium of studies selected in this

meta-analysis was the most comprehensive". Unfortunately, this does not mean anything, because as mentioned above you cannot mixt different clinical scenarios and dosages together.

Reply 6: Thank you for your comment and we agree with it. We have deleted the sentence.

Changes in the text: We deleted the sentence "the compendium of studies selected in this meta-analysis was the most comprehensive"((see Page 16, Line 16-17).

Comment 7: Line 202 – please indicate the meaning of "according to glutamine dosage".

Reply 7: Thank you for your comment. We replaced "according to glutamine dosage" by "in subgroup analysis conducted to estimate the effect of glutamine supplementation on specific dosages (above 0.5 g/kg/day, between 0.3 g/kg/day and 0.5 g/kg/day, below 0.3 g/kg/day)".

Changes in the text: We replaced "according to glutamine dosage" by "in subgroup analysis conducted to estimate the effect of glutamine supplementation on specific dosages (above 0.5 g/kg/day, between 0.3 g/kg/day and 0.5 g/kg/day, below 0.3 g/kg/day)" (see Page 16, Line 18-19).

Comment 8: Lastly, as you found no difference between Glutamine and control groups in terms of mortality, does this not mean that Glutamine addition is safe and can therefore be provided to patients?

Reply 8: Thank you for your comment. In our study, we found that there was no difference between glutamine and control groups in terms of mortality, but that doesn't mean that glutamine addition is safe and can therefore be provided to patients. Although supplemental glutamine shortened the length of mechanical ventilation among critically ill patients, there was no effect on hospital mortality, mortality at 28 days, mortality at 6 months, or ICU mortality. The effect of glutamine supplement was relatively safe for critically ill patients. However, we suggest that supplemental glutamine need not be routinely delivered to critically ill patients because of no effect on mortality in critically ill patients, the supplemental glutamine should be considered for severely burned patients due to the reduced hospital mortality we observed among this patient subgroup.

Changes in the text: We have modified our text as advised (see Page 19, Line 8-14).

Reviewer C

Comment 1: The presentation of results is poor.

Reply 1: Thank you for your comment and we agree with it. We have added the data

in results. Length of ICU stay, length of hospital stay, and nosocomial infections were included.

Changes in the text: We added some data (see Page 11, Line 8-21; Page 12, Line 1-10, 19-21; Page 13 Line 1-21; Page 14 Line 1-10, 12-14; Page 15 Line 1-7).

Comment 2: The discussion is limited to citing the works of others without critically addressing a personal opinion of the results that features, for example about the heterogeneity of the various studies (regarding doses and administration time) included in the meta-analysis. For this reason, what is written in the discussion is vague, and does not fit harmoniously in the context of the systematic review, therefore it is not a real discussion.

Reply 2: Thank you for your suggestions. We added "Glutamine is one of the most important antioxidants in human cells. In critically ill patients, plasma glutamine concentration may be decreased during intense immune cell activity. Lack of glutamine results in less expression of surface activation proteins on and production of cytokines, and induces apoptosis in these cells[17]. Glutamine supplement helps to maintain its plasma level and improve prognosis in patients with critical disease conditions" in discussion.

Changes in the text: We have modified our text as advised (see Page 15, Line 9-14).