PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis
AUTHORS	Kiss, Szabolcs; Németh, Dávid; Hegyi, Péter; Földi, Mária; Szakács, Zsolt; Erőss, Bálint; Tinusz, Benedek; Hegyi, Péter Jenő; Sarlós, Patrícia; Hussain, Alizadeh

VERSION 1 – REVIEW

REVIEWER	Wang, Ying-De First Affiliated Hospital of Dalian Medical University, Gastroenterology
REVIEW RETURNED	05-Aug-2020

GENERAL COMMENTS	Selective granulocyte-monocyte apheresis (GMA) therapy in IBD, especially in UC patients, was practiced in many conutries in the last two decades, and has been proven to be an effective and safe therapeutic option for the patients. However, the efficacy of GMA is debated because no significant difference in clinical remission rate was found by some authors when compared with a placebo. Therefore, this timely meta analysis of randomized controlled trials to assess the efficacy and safety of GMA as an adjunctive therapy in patients with UC reasonably addressed this controversy. In our own experiences, we found that the clinical remission rate was about 60% to 80% in UC patients depending upon the frequency of sessions performed. This paper was well written with logical discussions and conclusions.

REVIEWER	Naganuma, Makoto Kansai Medical University Hirakata Hospital
REVIEW RETURNED	13-Sep-2020

GENERAL COMMENTS	Thank you for giving me the opportunity to review the manuscript regarding meta-analysis for efficacy of granulocyte-monocyte apheresis to induce and maintain remission. I have no comment on the methods for meta-analysis of this study. However, most studies have small sample sizes and/or the quality of the studies is low except Sands' study. I think that the meta-analysis, which is a collection of such studies, may lead the conclusion in the wrong direction.

REVIEWER	Gubatan, John Stanford University School of Medicine, Gastroenterology and Hepatology
REVIEW RETURNED	14-Nov-2020

GENERAL COMMENTS	In this systematic review and meta-analysis, Kiss et al sought to determine the efficacy of granulocyte and monocyte apheresis as a adjunctive therapy for ulcerative colitis induction and maintenance. In this meta-analysis of 11 studies, the authors demonstrate that granulocyte and monocyte apheresis adjunct therapy was associated with increased odds of clinical remission during induction (N= 598 patients) and maintenance therapy (N= 71 patients). The authors address an interesting topic and the systematic review and meta-analysis was overall well-designed and reported. I have the following critiques and recommendations: -Title: "Granulocyte and monocyte apheresis is an excellent choice as an adjunctive therapy to induce and maintain remission in ulcerative colitis: A meta-analysis of randomized controlled trials." The use of "excellent choice" is subjective and should be avoided as the alternative to these ulcerative patients (only on mesalamine, 6MP/AZA) flaring would be to biologic escalation as standard of care which was not addressed by manuscript. Title should also clarify that this was "clinical" remission. -Abstract, Results: Results for outcome of clinical remission during induction and maintenance therapy should also include the I2 static to indicate heterogeneity along their odds ratios. -Abstract, Results: The authors should also state results from pooled adverse events in abstract as this was a secondary outcome. -Methods/Results: Table 1 should include inclusion criteria/definitions for ulcerative colitis disease activity. -Methods/Results: Did any of the included studies demonstrate change in objective markers of inflammation (CRP, fecal calprotectin), endoscopic inflammation scores (e.g. Mayo endoscopic scores), or histologic inflammation scores (e.g. Geboes scores) with granulocyte and monocyte apheresis adjunctive therapy. -Methods/Results: There was some heterogeneity with number of cycles of apharesis and time of induction response assessment (ranging from 12 days to 12 weeks). This may have impacted results. The authors should perform a sensitivity analysis restricting meta-analysis to only clinical remission induction assessed at 12 weeks as this was the most common time point. -Results/Discussion: The ulcerative colitis patients were on various medications (steroids, mesalamine/5-ASA, 6MP/AZA) in addition to apharesis adjunctive therapy. The authors should address this limitation and likely source of bias and heterogeneity in their results. -Results/Discussion: Among patients who were on apharesis adjunctive treatment for maintenance therapy, what was the median time to clinical relapse after last cycle of apharesis? -Results: Is there any data from included studies how effective granulocyte/monocyte apharesis is at actually reducing peripheral blood granulocyte and monocyte numbers in patients with ulcerative colitis after apharesis? If data is available, it would be interesting to include this as a pooled reduction in

	granulocyte/monocyte numbers post-treatment (expressed as standardized mean difference) as a secondary outcome in this meta-analysis. Did reduction in peripheral granulocyte/monocyte count correlate with rates of clinical remission/response in the included studies? -Discussion/Conclusions: The authors argue that apharesis adjunctive therapy is effective for induction and maintenance therapy in ulcerative colitis compared to standard of care alone. However, it is standard of care for ulcerative colitis patients who are flaring/have active disease only on mesalamine (5-ASA) or 6MP/AZA to be escalated to a biologic (anti-TNF agent, vedolizumab, ustekinumab, etc) . Is there any data on how granulocyte/monocyte apharesis compares to biologic therapy in ulcerative colitis? Starting biologics may be less invasive and more readily available compared to apharesis. The practical implications of apharesis versus biologics should be considered in the discussion.

REVIEWER	Gordon, Morris University of Central Lancashire School of Medicine, WELFARE, PROFESSIONALISM, TRANSITION AND CAREERS
REVIEW RETURNED	02-Dec-2020

GENERAL COMMENTS	This is a good review and I only have minor comments, although important Firstly, it is timely - our cochrane review is 8 years out of date and we are waiting for an approach from an interested team to take it on as a review?? The title is inappropriate - excellent choice should not be stated and it should be 'a systematic review and meta-analysis'. The risk of bias - minimization does not necessarily equate to high risk Details of other judgements are quite sparse and given risk of bias is quite poor i think more detail is detail I think the definitions of the outcomes need cliarification I wonder about the use of Odds ratio- I would think RR is more appropriate? My largest concern is GRADE I think the moderate judgements are too lax. I think the risk of bias is very serious - not just serious- for the remission outcome, there is imprecision due ot low numbers of dichotomous events - the three together I think should downgrade to Low certainty - this is key. I don't see the GRADE analysis has been used to guide discussion or conclusion? What about 'Apheresis may be more effective for induction of remission in UC (Low certainty).

	This language is useful and needs to follow through

REVIEWER	Nolan, J Northern Kentucky University, Mathematics & Statistics
REVIEW RETURNED	08-Jan-2021

GENERAL COMMENTS	I am reviewing only the statistical methods for the paper. The authors have followed (reasonably well, I believe) guidance for reporting items for systematic reviews and meta-analyses found here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2714657/ They have openly addressed potential for bias and provided Forest Plots in a usual format. I have two suggestions (and these are the only reason that I indicated minor revision rather than accept): 1. When addressing bias, if possible assess the potential direction of bias based on the flaws in the original studies. In other words, if you identify a study having a high risk of bias, based on what is learned from the publication can you address to any extent whether that bias would favor or disfavor the treatment (and/or how large it might be). 2. In conclusions, you have statistically significant difference, but that does not address clinical importance. Can you in some way take the odds-ratio CI's and use them to address clinical importance? Such an assessment also must connect back to original probability of event. Figure 2 for example, you have odds ratio of 1.28 to 2.91 for the overall analysis. What would these two numbers (and/or numbers in between) mean for patients and care, if they represented the truth?

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer 1:

• Selective granulocyte-monocyte apheresis (GMA) therapy in IBD, especially in UC patients, was practiced in many conutries in the last two decades, and has been proven to be an effective and safe therapeutic option for the patients. However, the efficacy of GMA is debated because no significant difference in clinical remission rate was found by some authors when compared with a placebo. Therefore, this timely meta analysis of randomized controlled trials to assess the efficacy and safety of GMA as an adjunctive therapy in patients with UC reasonably addressed this controversy. In our own experiences, we found that the clinical remission rate was about 60% to 80% in UC patients depending upon the frequency of sessions performed. This paper was well written with logical discussions and conclusions.

Author response: Thank you for comments. We appreciate the reviewer's assessment.

Comments from Reviewer 2:

• Thank you for giving me the opportunity to review the manuscript regarding meta-analysis for efficacy of granulocyte-monocyte apheresis to induce and maintain remission. I have no comment on the methods for meta-analysis of this study. However, most studies have small sample sizes and/or the quality of the studies is low except Sands' study. I think that the meta-analysis, which is a collection of such studies, may lead the conclusion in the wrong direction.

Author response: Thank you for comments. Although we agree that this is an important consideration, we think this study makes a valuable contribution to the field. As suggested by the Editors and Reviewer 5, we revised our limitations and the certainty of evidence. We hope that this will contribute to a critical approach to evaluating and interpreting our results.

Comments from Reviewer 3:

• In this systematic review and meta-analysis, Kiss et al sought to determine the efficacy of granulocyte and monocyte apheresis as a adjunctive therapy for ulcerative colitis induction and maintenance. In this meta-analysis of 11 studies, the authors demonstrate that granulocyte and monocyte apheresis adjunct therapy was associated with increased odds of clinical remission during induction (N= 598 patients) and maintenance therapy (N= 71 patients). The authors address an interesting topic and the systematic review and meta-analysis was overall well-designed and reported. I have the following critiques and recommendations:

Author response: Thank you for comments. We appreciate the reviewer's assessment.

• Title: "Granulocyte and monocyte apheresis is an excellent choice as an adjunctive therapy to induce and maintain remission in ulcerative colitis: A meta-analysis of randomized controlled trials." The use of "excellent choice" is subjective and should be avoided as the alternative to these ulcerative patients (only on mesalamine, 6MP/AZA) flaring would be to biologic escalation as standard of care which was not addressed by manuscript. Title should also clarify that this was "clinical" remission.

Author response: We appreciate your feedback. We removed the declarative and subjective part of the title. The new title also clarifies the type of remission. The current version title is: " Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis"

• Abstract, Results: Results for outcome of clinical remission during induction and maintenance therapy should also include the I2 static to indicate heterogeneity along their odds ratios.

Author response: Thank you for pointing this out. The revised abstract contains the results regarding heterogeneity.

• The authors should also state results from pooled adverse events in abstract as this was a secondary outcome.

Author response: Thank you for pointing this out. The revised abstract contains the results from pooled adverse events.

• Methods/Results: Did any of the included studies demonstrate change in objective markers of inflammation (CRP, fecal calprotectin), endoscopic inflammation scores (e.g. Mayo endoscopic scores), or histologic inflammation scores (e.g. Geboes scores) with granulocyte and monocyte apheresis adjunctive therapy.

Author response: Thank you for your comment. This is an interesting aspect. Relevant results for endoscopic scores and objective markers of inflammation are discussed in the revised manuscript. Unfortunately, most of the articles analysed papers come before the 2015 STRIDE program (https://pubmed.ncbi.nlm.nih.gov/26303131/) and before setting new unified therapeutic goals. In light of this, the implication for research have been supplemented with the following: "There is currently

evidence of induction and maintenance of clinical remission; however, the role of GMA in endoscopic and histological remission is currently unclear."

• Methods/Results: There was some heterogeneity with number of cycles of apharesis and time of induction response assessment (ranging from 12 days to 12 weeks). This may have impacted results. The authors should perform a sensitivity analysis restricting meta-analysis to only clinical remission induction assessed at 12 weeks as this was the most common time point.

Author response: Thank you for the suggestion. We considered performing this subgroup analysis to be a good idea. The proposed analysis (OR = 1.67, 95% CI = 1.12–2.49, p=0.012, I2 = 0.0%) is incorporated into the manuscript and the forest plot is attached.

• Results/Discussion: The ulcerative colitis patients were on various medications (steroids, mesalamine/5-ASA, 6MP/AZA) in addition to apharesis adjunctive therapy. The authors should address this limitation and likely source of bias and heterogeneity in their results.

Author response: Thanks for you for pointing this out. Regarding bias, out limitation section highlights this phenomenon: "Allowed concomitant therapies have differed among included studies; therefore, our estimates may have been subject to bias, as reflected by the grade of evidence (Supplementary Table 2)." In the case of the limitation because of statistical heterogeneity, our statement has been supplemented as requested: "…strongly limited by the high heterogeneity of studies. The most likely source of this is the heterogeneous nature of concomitant treatment…."

• Results/Discussion: Among patients who were on apharesis adjunctive treatment for maintenance therapy, what was the median time to clinical relapse after last cycle of apharesis?

Author response: Thank you for the comment. In addition to remission maintenance, an important aspect is whether this is maintained for longer. Only Maiden et al. provided information on that. Because this endpoint was not included in the study protocol, we incorporated this only into the discussion section of the original version of the manuscript to avoid selective outcome reporting. "Maiden et al. found that time to first relapse was significantly higher in patients receiving GMA (99±73 days vs. 161±44 days, p=0.0004)"

• Is there any data from included studies how effective granulocyte/monocyte apharesis is at actually reducing peripheral blood granulocyte and monocyte numbers in patients with ulcerative colitis after apharesis? If data is available, it would be interesting to include this as a pooled reduction in granulocyte/monocyte numbers post-treatment (expressed as standardized mean difference) as a secondary outcome in this meta-analysis. Did reduction in peripheral granulocyte/monocyte count correlate with rates of clinical remission/response in the included studies?

Author response: Thank you for the suggestion. In our opinion, this is an excellent idea, however, the publications do not contain any data on this, so we cannot incorporate it into either the qualitative or quantitative synthesis.

• The authors argue that apharesis adjunctive therapy is effective for induction and maintenance therapy in ulcerative colitis compared to standard of care alone. However, it is standard of care for ulcerative colitis patients who are flaring/have active disease only on mesalamine (5-ASA) or 6MP/AZA to be escalated to a biologic (anti-TNF agent, vedolizumab, ustekinumab, etc) . Is there any data on how granulocyte/monocyte apharesis compares to biologic therapy in ulcerative colitis? Starting biologics may be less invasive and more readily available compared to apharesis. The practical implications of apharesis versus biologics should be considered in the discussion.

Author response: Thank you for pointing this out. There is currently no evidence for this comparison. This is now addressed in the relevant section of the discussion. In this regard, limited data are available from recent studies suggesting that GMA may be beneficial in patients who no longer respond to biologics. This was also briefly incorporated into the revised manuscript.

Comments from Reviewer 4:

• This is a good review and I only have minor comments, although important Firstly, it is timely - our cochrane review is 8 years out of date and we are waiting for an approach from an interested team to take it on as a review??

Author response: We appreciate the reviewer's assessment.

• The title is inappropriate - excellent choice should not be stated and it should be 'a systematic review and meta-analysis'.

Author response: We appreciate your feedback. We changed our declarative title to:
" Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis"

• Details of other judgements are quite sparse and given risk of bias is quite poor i think more detail is detail

Author response: Thank you for pointing this out. We attached a supplementary document that contains a short summary followed by support for each of the decisions.

• I think the definitions of the outcomes need cliarification

Author response: Thank you for pointing this out. We added the following section to the revised manuscript: "Outcome criteria for clinical remission and clinical response were defined individually by the eligible articles. These criteria are presented in Table 1. Regarding safety, AEs reported by the individual article were used for the analyses in each case. No preliminary specification was made."

• I wonder about the use of Odds ratio- I would think RR is more appropriate?

Author response: Thank you for the suggestion. We consider it a good idea to calculate the relative risk instead of the odds ratio, because relative risk is a concept that people more intuitively understand as a measure of association. Despite the fact that these two terms are similar concepts, their information contents are different and as event rates increase, the two ratios diverge and can no longer be used interchangeably. Our main argument in favour of maintaining the odds ratio is the study protocol. We believe that changing the measure of association would introduce additional limitation by protocol deviation. Furthermore, our results would remain significant even after the calculation of the relative risk. Of course, this would show a smaller benefit due to the relationship between OR and RR. But, if the reviewer deems it necessary to make the change, we will perform the necessary analyses; however, this could take relatively long time.

• My largest concern is GRADE. I think the moderate judgements are too lax. I think the risk of bias is very serious - not just serious- for the remission outcome, there is imprecision due ot low numbers of dichotomous events - the three together I think should downgrade to Low certainty - this is key.

Author response: Thank you for the feedback. We share your concern. In order to avoid overestimation of the certainty of evidence, we revised the first assessment and changed all levels of

evidence to very low and low certainty by incorporating the suggestions.

• I don't see the GRADE analysis has been used to guide discussion or conclusion? What about 'Apheresis may be more effective for induction of remission in UC (Low certainty). This language is useful and needs to follow through

Author response: We think this is an excellent suggestion. Incorporating GRADE results helps the reader to put the results into context. We placed these results in the following places: "…Based on our results, addition of GMA may be more effective for induction of remission in UC compared to conventional therapy alone (very low certainty)…."; "…We found no significant difference between the two groups as regards AEs (very low certainty)..."; "Our study showed that the addition of GMA enhances the proportion of patients who can maintain their remission (low certainty)."

Comments from Reviewer 5:

• I am reviewing only the statistical methods for the paper. The authors have followed (reasonably well, I believe) guidance for reporting items for systematic reviews and meta-analyses found here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2714657/

Author response: Thank you for comments. We appreciate the reviewer's assessment.

• They have openly addressed potential for bias and provided Forest Plots in a usual format. I have two suggestions (and these are the only reason that I indicated minor revision rather than accept):
1. When addressing bias, if possible assess the potential direction of bias based on the flaws in the original studies. In other words, if you identify a study having a high risk of bias, based on what is learned from the publication can you address to any extent whether that bias would favor or disfavor the treatment (and/or how large it might be).

Author response: We appreciate your feedback. Risk of bias assessment is an essential part of all systematic reviews. However, the tools used for this share a common limitation. Some domains are highly evaluator-dependent and therefore subjective. In addition, the distance between categories (in Cochrane Risk of Bias Tool) is impossible to quantify. Of course, quantifying the possibility of publication bias is possible, e.g., quantification of funnel plot asymmetry with a statistical test. It should be emphasized that these tests require an adequate number of tests per endpoint (typically above 10). In our opinion, the most significant distortion may be caused by the lack of blinding in some cases. A recent meta-analysis (https://doi.org/10.1093/ecco-jcc/jjw004) pointed out that the placebo intervention is associated with improvements in clinical outcomes [response and remission] in both induction and maintenance trials. We expected to see a greater difference for studies without adequate blinding. In the case of induction, this was observed, with most high-risk studies being among the studies with higher benefit (Hanai et al. 2004, Bresci et al. 2008, Hanai et al. 2008). In the case of maintenance, this is more difficult to judge because the results are very similar and two of the three studies are high risk in terms of blinding. In summary, the possibility of bias overestimation is present, however, this was taken into account in the revision with respect to the GRADE assessment. The risk of bias domain has been changed from serious to very serious in the appropriate places, helping to assess the certainty of evidence more critically.

• 2. In conclusions, you have statistically significant difference, but that does not address clinical importance. Can you in some way take the odds-ratio CI's and use them to address clinical importance? Such an assessment also must connect back to original probability of event. Figure 2 for example, you have odds ratio of 1.28 to 2.91 for the overall analysis. What would these two numbers (and/or numbers in between) mean for patients and care, if they represented the truth?

Author response: Thank you for pointing this out. In our case, in addition to statistical significance, it shows that if the study were subjected to an infinite number of replicates, 95% of the results would fall within this interval. To make it easier for the reader to interpret our results, we briefly present a simplified interpretation through the example of remission induction in the discussion section. “This result (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I2 = 0.0%) implies that patients receiving GMA have higher odds of achieving clinical remission by between 28 and 191%.

VERSION 2 – REVIEW

REVIEWER	Gubatan, John Stanford University School of Medicine, Gastroenterology and Hepatology
REVIEW RETURNED	26-Jan-2021

GENERAL COMMENTS	The authors have adequately addressed the reviewers' and editors' critiques and have improved the manuscript. I support publication of this manuscript.

REVIEWER	Nolan, J Northern Kentucky University, Mathematics & Statistics
REVIEW RETURNED	10-Feb-2021

GENERAL COMMENTS	Only one additional comment in regard to what I said last time. The addition of "This result (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I2 = 0.0%) implies that patients receiving GMA have higher odds of achieving clinical remission by between 28 and 191%." is insufficient for addressing clinical impact. For that information to be clinically useful, the typical remission probability should also be reported (going from 0.1% to 0.128% would be very different from going from 20% to at least 25.6%). OR itself is just generally difficult for readers to understand but if you can go from that to actually talking about probability of clinical remission, now you have a concept that is a lot easier for everyone to understand. You could move to relative risk as suggested by another reviewer, but with either OR or RR, to put them into clinical perspective one still needs to know the underlying basic probability of the event. It's a better manuscript if you can incorporate that.

VERSION 2 – AUTHOR RESPONSE

Comments from Reviewer: 3, Dr. John Gubatan, Stanford University School of Medicine:

- The authors have adequately addressed the reviewers' and editors' critiques and have improved the manuscript. I support publication of this manuscript.

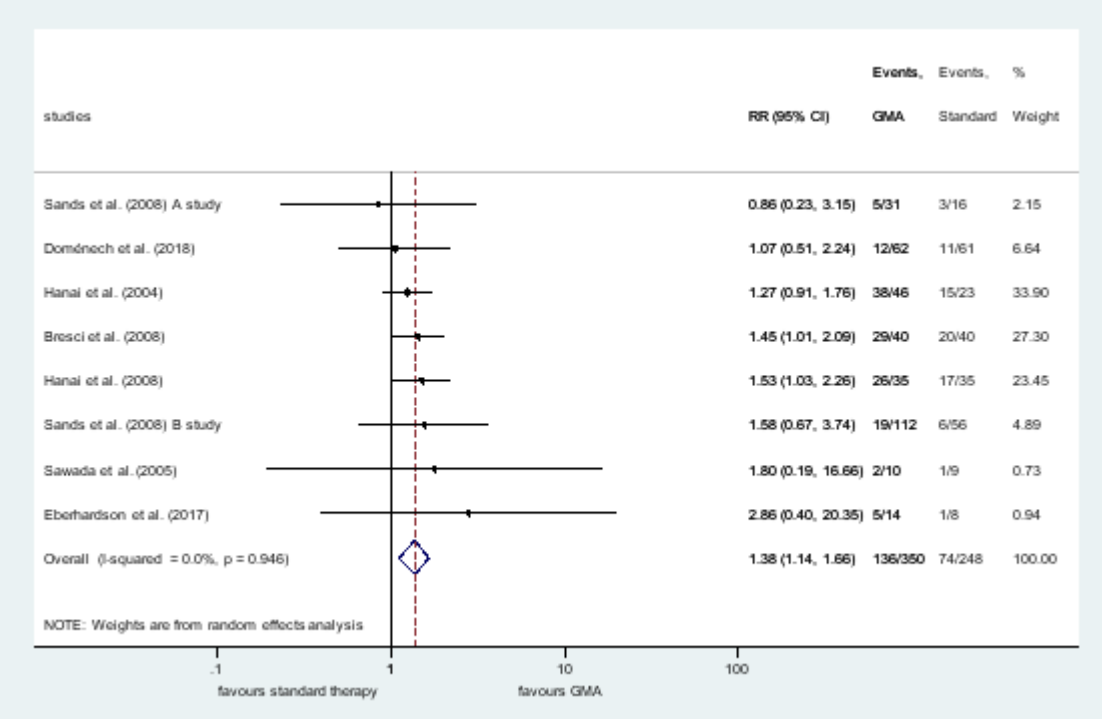
Author response: Thank you, we appreciate your feedback.

Comments from Reviewer: 5, Dr. J Nolan, Northern Kentucky University:

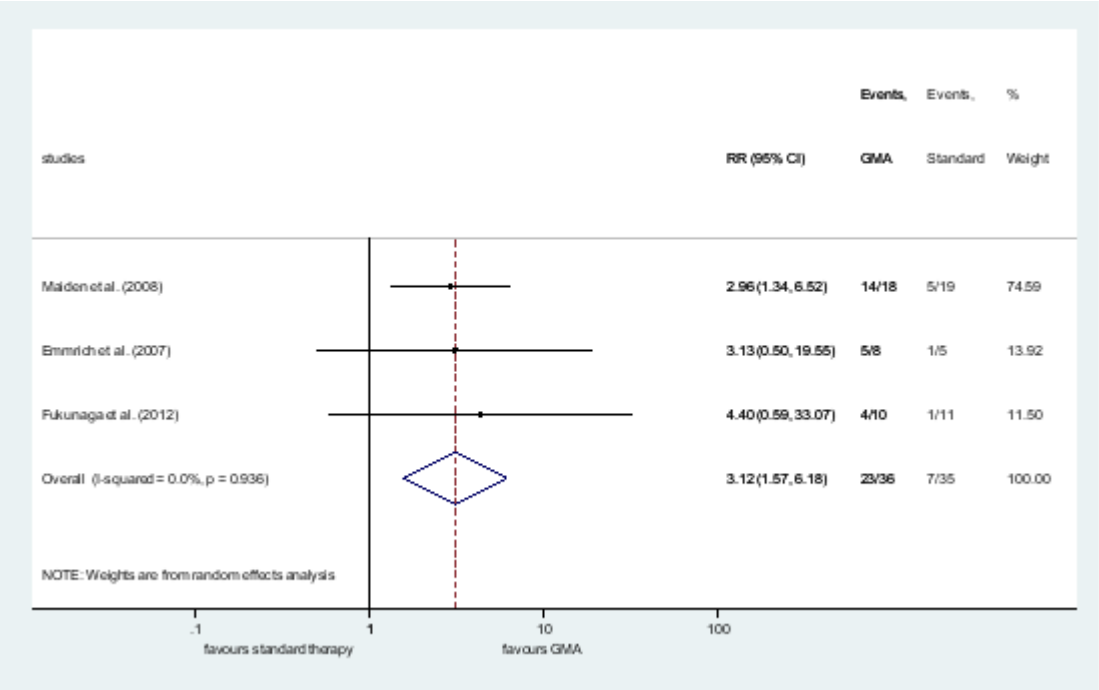
- Only one additional comment in regard to what I said last time. The addition of "This result (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I2 = 0.0%) implies that patients receiving GMA have higher odds of achieving clinical remission by between 28 and 191%." is insufficient for addressing clinical impact. For that information to be clinically useful, the typical remission probability should also be reported (going from 0.1% to 0.128% would be very different from going from 20% to at least 25.6%). OR itself is just generally difficult for readers to understand but if you can go from that to actually talking about probability of clinical remission, now you have a concept that is a lot easier for everyone to understand. You could move to relative risk as suggested by another reviewer, but with either OR or RR, to put them into clinical perspective one still needs to know the underlying basic probability of the event. It's a better manuscript if you can incorporate that.

Author response: Thank you for your valuable comments. As suggested, we added the requested content. Indeed, the extent of the change can only be interpreted correctly with the underlying basic probability of the event. We still consider it a good alternative to use RR instead of OR; however as OR was the prespecified measure in our study protocol, we would like to report our results in that way, if possible, to preserve transparency. After calculating RR, the conclusion would remain the same. Please find additional forest plots attached in this document. For clinical remission induction: RR = 1.38, 95% CI = 1.14–1.66, p=0.001, I^2 = 0.0%. For clinical remission maintenance: RR = 3.12, 95% CI = 1.57–6.18, p=0.001, I^2 = 0.0%.

The revised text reads as follows: ”In the eligible studies, clinical remission induction was achieved in 29.8% without adjunctive GMA therapy. Based on our analysis, addition of GMA may be more effective for induction of remission in UC compared to conventional therapy alone (very low certainty). This result (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I^2 = 0.0%) implies that patients receiving GMA have higher odds of achieving clinical remission by between 28 and 191%.”



RR for clinical remission induction



RR for clinical remission maintenance