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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

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1 Abstract

Objective: The goal of treatment in ulcerative colitis (UC) is to induce and maintain remission. The addition of granulocyte and monocyte apheresis (GMA) to conventional therapy may be a promising therapeutic alternative. In this meta-analysis, we aimed to assess the efficacy and safety profile of GMA as an adjunctive therapy.

Design: Systematic review and meta-analysis.

Methods: We searched four databases (MEDLINE, Embase, Web of Science, and Cochrane Central Register of Controlled Trials) for randomized or minimized controlled trials which discussed the impact of additional GMA therapy on clinical remission induction and clinical remission maintenance compared to conventional therapy alone. Primary outcome were clinical remission induction and maintenance, secondary outcomes were adverse events (1) and steroid-sparing effect. Odds ratios (OR) with 95% confidence intervals were calculated. Trial Sequential Analyses (TSA) were performed to adjusts for the risk of random errors in meta-analyses.

Results: A total of eleven studies were eligible for meta-analysis. GMA was clearly demonstrated to induce and maintain clinical remission more effectively than conventional therapy alone (598 patients: OR: 1.93, CI: 1.28–2.91, p=0.002 for induction; 71 patients: OR: 8.34, CI: 2.64–26.32, p<0.001 for maintenance).

Conclusion: GMA appears to be more effective as an adjunctive treatment in inducing and maintaining remission in UC patients than conventional therapy alone.

Protocol registration number: PROSPERO CRD42019134050.

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2 Article Summary

Strengths and limitations of this study

- This meta-analysis showed for the first time that GMA remarkably improves clinical remission maintenance compared with standard therapy alone in patients with UC.
- Grading of Recommendations Assessment, Development and Evaluation approach was applied to appraise the certainty of evidence.
- Our results are limited by the relatively low number of patients.
- To address the limitation by the number of included patients and to control both type I and type II errors, Trial Sequential Analyses have been performed.

3 Introduction

Ulcerative colitis (UC) is one of two major types of inflammatory bowel disease (IBD). The incidence of this disease varies from nine to 20 cases per 100 000 person-years (2). UC is a lifelong illness that has a profound impact on patients. The primary goal of treatment is to achieve and maintain remission, thereby preventing colectomy and colorectal neoplasms and ensuring an acceptable quality of life (3). The choice of treatment for patients with UC is tied to the clinical and endoscopic severity of the disease along with the frequency and severity of relapses. Patients with no response to conventional therapies, especially to corticosteroids and immunosuppressive agents, are common candidates for biological treatments and/or surgery. However, both of these options are challenged by the high costs and incidence of side-effects and complications.

Patients with UC usually have a raised level of granulocytes, and, in the case of an active disease, the mucosa of the bowel is infiltrated by a large number of granulocytes and macrophages. These leukocytes release degradative enzymes and proinflammatory cytokines, which lead to further inflammation of the bowel. Based on the hypothesis that a reduction of activated granulocytes and monocytes/macrophages may be beneficial, granulocyte-monocyte apheresis (GMA) was proposed as a strategy to promote remission in active UC (4). GMA is a novel non-pharmacological treatment tool for patients with UC, comprising an extra-corporeal absorptive circuit, which decreases inflammatory cytokines and upregulates regulatory T cells. Despite its high cost, GMA seems to have a good safety profile (4).

However, data on the efficacy of GMA are still debated. The first studies published in Japan showed remission or response rates of up to 60–80% (1, 5, 6). Sands et al. reported a study with a large number of patients comparing GMA to a placebo, and they found no significant difference in terms of clinical response (7). This substantial difference between studies could be explained by the heterogeneity of patients' characteristics, most probably by the varying severity and extent of the disease.

A large proportion of patients require long-term, high-dose steroid treatment, which often results in severe side-effects impairing patients' quality of life. If addition of GMA can reduce the dose of corticosteroids, the risk of steroid-induced adverse events (AEs) could be minimized. Therefore, it is also essential to evaluate the steroid-sparing effects of GMA (8). Beyond the induction of remission and the impact on steroid requirement, the role of GMA in maintaining remission is unclear (9). The aim of our study was to assess the role of GMA in the induction and maintenance of clinical remission in UC and to evaluate the potential steroid-sparing effect of the therapy.

4 Methods

The meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (10). The review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews (CRD42019134050).

4.1 Search strategy

The systematic literature search was conducted by two independent reviewers (KS and FM) in MEDLINE (via PubMed), EMBASE, the Cochrane Library (CENTRAL), and the Web of Science for studies published up to 5th March 2019. The search query in each database was based on PICO components combined with Boolean operators: (gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher*

OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND ("inflammatory bowel disease" OR "ulcerative colitis") AND (random*).

4.2 Eligibility criteria

General criteria: a randomized controlled trial (RCT) or minimized controlled trial (This type of sequence generation is considered to be nearly equivalent to being random) (11); only full-text articles were included.

Specific criteria for clinical remission induction: patients with active UC (*Population*$_1$), standard therapy for remission induction and GMA as an adjunctive therapy (*Intervention*$_1$), and standard therapy for remission induction (*Comparison*$_1$); *Outcomes*$_1$: clinical response rate and clinical remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.[12, 13]

Specific criteria for clinical remission maintenance: patients with UC in clinical remission induced by GMA (*Population*$_2$), standard therapy for remission maintenance and GMA as an adjunctive therapy (*Intervention*$_2$), and standard therapy for remission maintenance (*Comparison*$_2$); *Outcomes*$_2$: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

The titles of the studies were screened based on predefined criteria, and the relevant studies were selected for abstract review. If the abstract was found to be appropriate, the full text of the article was studied. The decision to include a study in the meta-analysis was based on an independent assessment by the two reviewers and eventually by consensus for resolution of any disagreements. Reference lists in included studies and reviews on this topic were searched for additional studies. Publications citing the included studies were also screened in the Google Scholar academic search engine.

4.3 Data extraction

The two investigators (KS and FM) reviewed the articles independently and extracted data into a standardized data collection form (discrepancies were resolved based on consensus). For the selected studies, characteristics were extracted, including publication year, country, number of centres, number of patients, and study design. In addition, patient characteristics (age, sex, and extent of disease), details of therapy (concomitant medication, volume of GMA, number of GMA cycles, and duration of treatment), and main outcomes (number of patients with clinical improvement/response, number of patients achieving clinical remission, number of patients with maintained remission, and number of AEs) were also extracted.

4.4 Risk of bias assessment

The Cochrane Risk of Bias Tool was used by the two independent investigators (KS and FM) to assess the quality of the studies included. Any disagreement was resolved based on consensus (12). Major domains of quality assessment were the following:

1. Random sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)
5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)

7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials, and imputation of intention-to-treat (ITT) analysis)

4.5 Statistical analysis

The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR) with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered statistically significant (except for heterogeneity, for which a p value <0.10 was considered significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR, WMD, and weights are presented in forest plots. The random-effects model was used to pool effect sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating Higgins' I^2 indicator.[(13, 14)6] The Q statistics were computed as the squared deviations from the pooled effect of the weighted sum of individual study effects, with the weights being used in the pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees of freedom (where k was the number of studies). The I^2 index corresponds to the percentage of the total variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–60%), substantial (50–90%), and considerable (75–100%).[(11)] Subgroup analysis was performed as described in the study protocol if a sufficient number of studies was available. Funnel plots were used to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal information size (OIS). This methodology combines an information size with the threshold of statistical significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).

4.6 Quality of evidence

The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality of evidence for each outcome (15, 16). Disagreements were resolved by consensus.

5 Results

5.1 Search and selection

The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After screening and assessment for eligibility, eleven full-text articles containing one minimized controlled trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active UC, and three studies contained data on patients with UC in clinical remission.

5.2 Characteristics of the studies included

The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients received GMA, and 248 were in control groups. All the participants had active UC and were treated with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received standard of care added to the intervention/comparator.

Both GMA and control were added to conventional treatment. In terms of main outcomes, the studies investigated the rate of clinical remission and clinical response. Investigators assessed the activity of

UC with either the Mayo score or CAI. One study required steroid-free remission to regard cases as being in clinical remission.

In the case of clinical remission maintenance, all the studies were randomized controlled trials. A total of 71 participants (mean: 24, ranging from 13 to 37) were included in this meta-analysis: 36 patients received GMA, and 35 were in control groups. All the participants had ulcerative colitis in remission and were treated with Adacolumn® or Cellsorba®. One trial evaluated GMA vs sham control (24) and two trials assessed GMA compared to standard therapy alone (9, 25). Both GMA and sham control were added to conventional treatment. In terms of main outcome, the studies investigated the rate of clinical relapse.

Three studies also reported on the steroid-sparing effect of GMA (9, 17, 22).

5.3 Risk of bias assessment

A summary of risk of bias assessment is shown in **Supplementary Figure 1 and Supplementary Figure 2**. One study was graded at a high risk of selection bias because it used minimization for sequence generation (17). Three unblinded studies were at high risk of performance bias (19, 22, 25). Because of the nature of the intervention, four studies which lacked a description of the blinding process were interpreted as having a high risk of bias (18, 21, 23, 24). As regards assessment blinding, two unblinded studies were judged to be at high risk of bias (19, 25). Two studies were deemed as having a high risk of other bias; although they used ITT analysis, they considered subjects who left the study as a treatment failure that may lead to bias (7).

5.4 Efficacy and safety of GMA in clinical remission induction

Seven randomized and one minimized controlled trial evaluated clinical remission induction. GMA therapy was associated with a better clinical response rate compared to the control group (OR = 2.03, 95% CI = 1.36–3.01, p<0.001, I^2 = 8.4%) (**Supplementary Figure 3**). Patients undergoing GMA therapy had a higher remission rate compared to standard therapy without GMA (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I^2 = 0.0%) (**Figure 2**). Sub-group analyses were performed based on activity indices and number of GMA cycles. No difference was found between the two groups in studies assessing UC with the Mayo score (OR = 1.34, 95% CI = 0.74–2.43, p=0.334, I^2 = 0.0%), but the remission induction was more successful in studies using CAI for assessment (OR = 2.70, 95% CI = 1.52–4.79, p=0.001, I^2 = 0.0%) (**Supplementary Figure 4**). A significant difference was found in studies using five cycles compared to the control (OR = 2.78, 95% CI = 1.17–6.60, p=0.021, I^2 = 0.0%) and more than five cycles compared to standard therapy alone (OR = 1.73, 95% CI = 1.08–2.77, p=0.022, I^2 = 0.0%). There was no statistically significant difference in the number of AEs (p=0.135) (**Supplementary Figure 5**). No statistically significant steroid-sparing effect was detected among patients with active UC (p=0.080). A list of reported AEs is presented in **Supplementary Table 1**.

5.5 Efficacy and safety of GMA in clinical remission maintenance

Three randomized clinical trials evaluated the clinical remission rate in remitting UC induced by GMA. Patients receiving GMA had a higher rate of clinical remission maintenance (OR = 8.34, 95% CI = 2.64–26.32, p<0.001, I^2 = 0.0%) (**Figure 3**). Due to lack of data, the rate of AEs could not be assessed in this population.

5.6 Trial Sequential Analysis

Based on a TSA, the cumulative Z curve crossed the trial sequential significance boundary as regards clinical remission induction and clinical remission maintenance (power=80.0%; alpha=5.0%) (**Supplementary Figure 6)**. Moreover, clinical remission maintenance exceeded the required meta-analysis sample size, possibly suggesting that further clinical trials are not required. A TSA for AEs and steroid-sparing effects could not be carried out due to insufficient information size.

5.7 Quality of evidence

The GRADE analysis rated the quality of evidence for primary and secondary outcomes at a very low to moderate level. GRADE evidence profile is shown in **Supplementary Table 2**.

6 Discussion

The main goal of care is to achieve and maintain remission of UC. This condition is usually treated by a step-up approach, during which treatments are switched or additional treatment is administered to optimize current therapy. There are several therapeutic agents to slow down the clinical activity of UC. Corticosteroids, 5-aminosalicylates, immunosuppressive agents, and tumour necrosis alpha-inhibitors are commonly used, and new therapeutic targets, such as anti-adhesion molecules and anti-interleukins, are emerging. Despite these multiple therapeutic options, there is still a need to expand the scope of treatment methods due to possible development of intolerance or resistance to current treatments. After running out of treatment options, surgical therapy is frequently the last remaining option for patients. GMA is a novel non-pharmacologic treatment option for active and remitting UC, by which activated granulocytes and monocytes are removed from the circulation. These cells may contribute to the pathogenesis of UC.

Guidelines describing the role of GMA in UC are in agreement on the potential beneficial effect and favourable safety profile. They also agree that there is insufficient evidence in this field of practice (26, 27).

To our knowledge, the first report on the efficacy of GMA in UC was published in Japan in 2001 (28). This study found a considerably high remission rate with only five sessions of GMA in patients refractory to conventional drug therapy. Subsequent studies from the early 21st century had similar results (29-31). In 2008, Sands et al. failed to prove a significant difference in clinical remission rate between GMA and a placebo on a relatively large population (7). However, this study was not free of attrition bias; a high proportion of patients were lost to follow-up. Three systematic reviews and meta-analyses have been conducted in this field so far (32-34). All of them have agreed on the benefit of GMA in clinical remission induction, and they pointed out the necessity for more trials with a rigorous and clear design to further narrow the focus on specific patient groups. These studies used one to three databases for a systematic search and selection.

In our current meta-analysis, a broader literature search was carried out, and the role of GMA in clinical remission maintenance was assessed. Our work supported the hypothesis that GMA improves the rates of clinical response and clinical remission in patients with UC. It should be noted that response and remission rates defined by symptom scores should be cautiously interpreted because they also include subjective elements, such as overall physician judgement on disease activity. A few recent retrospective and prospective studies have suggested certain prognostic factors in the therapeutic response (35-37). It seems that younger patients respond better to GMA therapy, whereas gender and

smoking status showed no difference in response to treatment (35). Yokoyama et al. found that shorter duration of UC and lower cumulative corticosteroid dose are associated with a higher efficacy rate (36). In their study, patients who received GMA treatment immediately after relapse were the best responders. It would be advisable to conduct further research to identify subgroups of UC where patients benefit the most from GMA (38).

To date, there is no uniformly accepted GMA regimen. There are RCTs to compare a ten-cycle and a five-cycle GMA regimen. Dignass et al. and Ricart et al. found similar remission rates between ten and five cycles (46% vs. 36%, p=0.479; 35.7% vs 45.5%, p>0.05, respectively) (38, 39). The latter study also showed a steroid-sparing effect in the group receiving ten cycles of GMA. Sakuraba et al. found that an improved remission rate is associated with intensive GMA (54.0% vs 71.2%, p=0.029 in five- and ten-cycle regimens, respectively) (40). In our meta-analysis, the number of GMA cycles varied among studies as well. We assessed the efficacy of GMA based on the two main regimens in previous trials. Both groups showed a benefit of adding GMA to the therapy compared to standard treatment alone.

We found no significant difference between the two groups as regards AEs. Further studies are called for to provide a higher level of evidence on this topic. They would be particularly important for specific subgroups where the safety profile is of paramount importance, such as in cytomegalovirus infection, adolescence, and pregnancy. Clinical trials should also target these populations because fewer therapeutic options are available for them and the safety profile of GMA seems favourable compared to other treatments.

As with any therapeutic option, cost-effectiveness should also be considered. The cost of GMA is much higher compared to regular medication, such as corticosteroids, but GMA could be cost-effective in the long term. The use of GMA may reduce the cost of medical services, hospitalization, and surgery in the long term. Nevertheless, GMA's safety profile is in sharp contrast to multiple severe AEs associated with conventional pharmacologics and biologics.

To our knowledge, this is the first meta-analysis to assess the role of GMA in UC remission maintenance. Our study showed that the addition of GMA enhances the proportion of patients who can maintain their remission. Fukunaga et al. and Emmrich et al. enrolled clinically active UC patients based on CAI (9, 24). After successful induction therapy with the inclusion of GMA, patients achieving clinical remission were allocated to groups with and without GMA treatment for remission maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin, which is considered as a risk factor of relapse (25). This study differs from the previous two in the fact that they enrolled an asymptomatic population regardless of how patients achieved remission. The two studies recruiting patients with active UC detected no statistically significant difference between study arms in time to first relapse; however, it must be noted that in one of these studies, all the patients became steroid-free in the GMA group (9). 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). Despite our very promising results, these findings are limited by the amount of available data. More randomized controlled trials are necessary in this area to strengthen our results. This study has some potential limitations. 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**). Moreover, our funnel plots showed symmetry by visual assessment, but publication bias still cannot be ruled out because of the low number of included studies. Side-effects and safety data were not uniformly reported in most of the publications under analysis, according to the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines (41). Furthermore, this result is strongly limited by the high heterogeneity of studies. All in all, GMA seems to be a reasonable therapeutic option, but finding its

exact place to treat UC demands further research. A particularly promising area could be remission maintenance.

6.1 Conclusion

Implications for practice: The results support the hypothesis that patients with active UC have a better chance of remission if GMA is administered as an adjunctive therapy. As regards the frequency of AEs, we found no statistically significant difference between the two groups. With regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

Implications for research: Further studies are required to select patients who may benefit the most from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its role in remission induction. If GMA is proven to be safe and effective, cost-effectiveness studies will also be worthwhile in the future.

7 Data availability statement

The data that support the findings of this study are available from the corresponding author, [A.H.], upon reasonable request.

8 Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

9 Author contributions

S.K.: preparation of the draft of the manuscript, selection of studies, data extraction, risk of bias assessment; G.N.: statistical analysis, preparation of the standardized data collection sheet; P.H.: substantial contribution in study design; M.F.: selection of studies, data extraction; F.D.: selection of studies, data extraction; B.N. data extraction, risk of bias assessment; M.F.J.: preparation of the standardized data collection sheet, stylistic and grammatical revision of the manuscript; K.O.: risk of bias assessment, stylistic and grammatical revision of the manuscript; Z.M.: expert in the field of anaesthesiology and intensive therapy, substantial contribution in study design and interpretation of data, preparation of the manuscript; N.Z.: substantial contribution in study design; A.P.: preparation of the study protocol; P.J.H.: preparation of the standardized data collection sheet, stylistic and grammatical revision of the manuscript; Z.S.: participation in the design of the study and its coordination; P.G.: provided revisions to the scientific content of the manuscript; B.E.: provided revisions to the scientific content of the manuscript; A.H.: expert in the field of haematology, substantial contribution in study design and interpretation of data, preparation of study protocol and the first draft of the manuscript.

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11 Conflict of interest

Authors do not have any conflicts of interest to declare.

12 Compliance with Ethical Standards

This study was prepared in accordance with the Committee on Publication Ethics (COPE) guidelines to respect third parties rights such as copyright and/or moral rights. Ethical approval was not required to conduct this project as data is not individualized and primary data was not collected.

13 Abbreviations

AE, adverse events; clinical activity index, CAI; confidence interval, CI; granulocyte and monocyte apheresis, GMA; inflammatory bowel disease, IBD; OR, odds ratio; RCT, randomized controlled trial; TSA, trial sequential analysis; UC, ulcerative colitis; weighted mean difference, WMD.

14 References

1. Yamamoto T, Umegae S, Matsumoto K. Long-term clinical impact of early introduction of granulocyte and monocyte adsorptive apheresis in new onset, moderately active, extensive ulcerative colitis. J Crohns Colitis. 2012;6(7):750-5.

2. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. The Lancet. 2012;380(9853):1606-19.

3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis. The Lancet. 2017;389(10080):1756-70.

4. Saniabadi AR, Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H. Treating inflammatory bowel disease by adsorptive leucocytapheresis: a desire to treat without drugs. World J Gastroenterol. 2014;20(29):9699-715.

5. Matsumoto T, Fukunaga K, Kamikozuru K, Tozawa K, Yokoyama Y, Kusaka T, et al. Cytapheresis as a Non-Pharmacological Therapy for Inflammatory Bowel Disease. Transfus Med Hemother. 2008;35(1):18-23.

6. Yamamoto T. The role of granulocyte and monocyte apheresis in inflammatory bowel disease. J Crohns Colitis. 2013;7(3):e114.

7. Sands BE, Sandborn WJ, Feagan B, Lofberg R, Hibi T, Wang T, et al. A randomized, double-blind, sham-controlled study of granulocyte/monocyte apheresis for active ulcerative colitis. Gastroenterology. 2008;135(2):400-9.

8. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Treatment of patients with acute ulcerative colitis: conventional corticosteroid therapy (MP) versus granulocytapheresis (GMA): a pilot study. Dig Liver Dis. 2007;39(5):430-4.

9. Fukunaga K, Yokoyama Y, Kamokozuru K, Nagase K, Nakamura S, Miwa H, et al. Adsorptive granulocyte/monocyte apheresis for the maintenance of remission in patients with ulcerative colitis: a prospective randomized, double blind, sham-controlled clinical trial. Gut Liver. 2012;6(4):427-33.

10. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

11. Hoboken. Cochrane Handbook for Systematic Reviews of Interventions: Wiley; 2008.

12. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

13. Cochran WG. The Combination of Estimates from Different Experiment. Biometrics. 1954;10:101-29.

14. Julian P T Higgins SGT, Jonathan J Deeks, Douglas G Altman. Measuring inconsistency in meta-analyses. British Medical Journal. 2003;327:557-60.

15. Schünemann H, Brożek J, Guyatt G, Oxman A, editors. GRADE handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The GRADE Working Group, 2013. Available from guidelinedevelopment.org/handbook..

16. Schünemann H, Brożek J, Guyatt G, A O. GRADE handbook for grading quality of evidence and strength of recommendations: The GRADE Working Group, 2013; 2013.

17. Sawada K, Kusugami K, Suzuki Y, Bamba T, Munakata A, Hibi T, et al. Leukocytapheresis in ulcerative colitis: results of a multicenter double-blind prospective case-control study with sham apheresis as placebo treatment. Am J Gastroenterol. 2005;100(6):1362-9.

18. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Granulocytapheresis versus methylprednisolone in patients with acute ulcerative colitis: 12-month follow up. J Gastroenterol Hepatol. 2008;23(11):1678-82.

19. Domenech E, Panes J, Hinojosa J, Annese V, Magro F, Sturniolo GC, et al. Addition of Granulocyte/Monocyte Apheresis to Oral Prednisone for Steroid-dependent Ulcerative Colitis: A Randomized Multicentre Clinical Trial. J Crohns Colitis. 2018;12(6):687-94.

20. Eberhardson M, Karlen P, Linton L, Jones P, Lindberg A, Kostalla MJ, et al. Randomised, Double-blind, Placebo-controlled Trial of CCR9-targeted Leukapheresis Treatment of Ulcerative Colitis Patients. J Crohns Colitis. 2017;11(5):534-42.

21. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Kageoka M, et al. Intensive granulocyte and monocyte adsorption versus intravenous prednisolone in patients with severe ulcerative colitis: an unblinded randomised multi-centre controlled study. Dig Liver Dis. 2008;40(6):433-40.

22. Hanai H, Watanabe F, Yamada M, Sato Y, Takeuchi K, Iida T, et al. Adsorptive granulocyte and monocyte apheresis versus prednisolone in patients with corticosteroid-dependent moderately severe ulcerative colitis. Digestion. 2004;70(1):36-44.

23. Nakamura T, Kawagoe Y, Matsuda T, Ueda A, Ueda Y, Takahashi Y, et al. Effect of granulocyte and monocyte adsorption apheresis on urinary albumin excretion and plasma endothelin-1 concentration in patients with active ulcerative colitis. Blood Purif. 2004;22(6):499-504.

24. Emmrich J, Petermann S, Nowak D, Beutner I, Brock P, Klingel R, et al. Leukocytapheresis (LCAP) in the management of chronic active ulcerative colitis--results of a randomized pilot trial. Dig Dis Sci. 2007;52(9):2044-53.

25. Maiden L, Takeuchi K, Baur R, Bjarnason I, O'Donohue J, Forgacs I, et al. Selective white cell apheresis reduces relapse rates in patients with IBD at significant risk of clinical relapse. Inflamm Bowel Dis. 2008;14(10):1413-8.

26. Leukapheresis for inflammatory bowel disease: Interventional procedures guidance 2005 [Available from: https://www.nice.org.uk/guidance/ipg126.

27. Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, et al. Evidence-based clinical practice guidelines for inflammatory bowel disease. J Gastroenterol. 2018;53(3):305-53.

28. Shimoyama T, Kimura K, Hiwatashi N, Matsueda K, Munakata A, Asakura H, et al. Safety and Efficacy of Granulocyte and Monocyte Adsorption Apheresis in Patients With Active Ulcerative Colitis: A Multicenter Study. Journal of Clinical Apheresis. 2001;16:1-9.

29. Kanke K, Nakano M, Hiraishi H, Terano A. Clinical evaluation of granulocyte/monocyte apheresis therapy for active ulcerative colitis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2004;36(12):811-7.

30. Kim HJ, Kim JS, Han DS, Yang SK, Hahm KB, Lee WI, et al. [Granulocyte and monocyte adsorption apheresis in Korean conventional treatment-refractory patients with active ulcerative colitis: a prospective open-label multicenter study]. The Korean journal of gastroenterology = Taehan Sohwagi Hakhoe chi. 2005;45(1):34-44.

31. Shimoyama T, Sawada K, Hiwatashi N, Sawada T, Matsueda K, Munakata A, et al. Safety and efficacy of granulocyte and monocyte adsorption apheresis in patients with active ulcerative colitis: a multicenter study. Journal of clinical apheresis. 2001;16(1):1-9.

32. Habermalz B, Sauerland S. Clinical effectiveness of selective granulocyte, monocyte adsorptive apheresis with the Adacolumn device in ulcerative colitis. Dig Dis Sci. 2010;55(5):1421-8.

33. Thanaraj S, Hamlin PJ, Ford AC. Systematic review: granulocyte/monocyte adsorptive apheresis for ulcerative colitis. Aliment Pharmacol Ther. 2010;32(11-12):1297-306.

34. Yoshino T, Nakase H, Minami N, Yamada S, Matsuura M, Yazumi S, et al. Efficacy and safety of granulocyte and monocyte adsorption apheresis for ulcerative colitis: a meta-analysis. Dig Liver Dis. 2014;46(3):219-26.

35. Imperiali G, Amato A, Terpin MM, Beverina I, Bortoli A, Devani M, et al. Granulocyte-Monocyte Apheresis in Steroid-Dependent, Azathioprine-Intolerant/Resistant Moderate Ulcerative Colitis: A Prospective Multicenter Study. Gastroenterol Res Pract. 2017;2017:9728324.

36. Yokoyama Y, Kawai M, Fukunaga K, Kamikozuru K, Nagase K, Nogami K, et al. Looking for predictive factors of clinical response to adsorptive granulocyte and monocyte apheresis in patients with ulcerative colitis: markers of response to GMA. BMC Gastroenterology. 2013;13(27).

37. Tanaka T, Sugiyama S, Goishi H, Kajihara T, Akagi M, Miura T. Treatment of children and adolescents with ulcerative colitis by adsorptive depletion of myeloid lineage leucocytes as monotherapy or in combination with low dose prednisolone after failure of first-line medications. BMC Gastroenterology. 2013;13(130).

38. Dignass AU, Eriksson A, Kilander A, Pukitis A, Rhodes JM, Vavricka S. Clinical trial: five or ten cycles of granulocyte-monocyte apheresis show equivalent efficacy and safety in ulcerative colitis. Aliment Pharmacol Ther. 2010;31(12):1286-95.

39. Ricart E, Esteve M, Andreu M, Casellas F, Monfort D, Sans M, et al. Evaluation of 5 versus 10 granulocyteaphaeresis sessions in steroid-dependent ulcerative colitis: A pilot, prospective, multicenter, randomized study. World Journal of Gastroenterology. 2007;13(15):2193-7.

40. Sakuraba A, Motoya S, Watanabe K, Nishishita M, Kanke K, Matsui T, et al. An open-label prospective randomized multicenter study shows very rapid remission of ulcerative colitis by

intensive granulocyte and monocyte adsorptive apheresis as compared with routine weekly treatment. Am J Gastroenterol. 2009;104(12):2990-5.

41. Bhatt A. International Council for Harmonisation E6(R2) addendum: Challenges of implementation. Perspectives in Clinical Research. 2017;8(4):162-6.

15 Figures and tables

Figure 1: PRISMA flow chart representing the process of the study search and selection

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Table 1: Characteristics of included studies

Supplementary Figure 1: Risk of bias assessment on study level in studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 2: Risk of bias assessment across studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 3: Forest plot of studies comparing clinical remission induction or clinical improvement between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 4: Subgroup analysis based on criteria of remission in studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 5: Forest plot of studies comparing frequency of adverse events between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 6: Results of Trial Sequential Analysis. A: clinical remission induction, B: clinical remission maintenance, C: Clinical remission induction based on remission criteria, D: Clinical remission induction or clinical improvement

Supplementary Table 1: List of reported adverse events.

Supplementary Table 2: Certainty of evidence by GRADE approach

Table 1: Characteristics of included studies

*** All patients received standard of care added to investigator/comparator. 1: one patient was excluded from analysis because of protocol deviations; 2: one patient was excluded from analysis because of protocol deviations; 3: one patient was excluded due to failure to return blood from the column; 4: minimization may be implemented without a random element, and this is considered to be equivalent to being random. Abbreviations: GMA=granulocyte/monocyte apheresis; n= number; Rem=clinical remission; Res=clinical response; CAI=Clinical Activity Index; EI=Endoscopic Index; 5-ASA=5-aminosalicylic acid; AZA=azathioprine; PSL=prednisolone; SAS=sulfasalazine; 6-MP=6-mercaptopurine; PSN=prednisone**

Clinical remission induction											
Study Name	Country / Setting	N⁰ of cycles (n)	Randomization*	N⁰ of patients analyzed (n)	Patients achieving Res		Patients achieving Rem		Time of assess-ment	Outcome criteria (Rem and Res)	Concomitant medication
					%	n	%	n			
Bresci 2008	single center in Italy	5	GMA	40	92.5	37	72.5	29	5 weeks	Rem= CAI<6; EI<4 Res= CAI<6; EI>4	oral 5-ASA
			steroid	40	65.0	26	50.0	20			
Doménech 2018	39 centers in Austria, Germany, Italy, Portugal, Spain	7	GMA+steroid	62 [1]	58.1	36	19.4	12	12 weeks	Rem= Mayo ≤2 and no steroid use; Res=Mayo score decrease ≥3 or at least 30% from baseline	stable dose AZA and PSL were allowed if started before randomization
			steroid	61 [2]	49.2	30	18.0	11			
Eberhardson 2017	single center in Sweden	5	GMA	14	57.1	8	35.7	5	12 days	Rem= Mayo score ≤3, Res= Mayo score decrease ≥3 or at least 30% from baseline	stable dose of steroid; 5-ASA and/or thiopurines were allowed
			sham	8 [3]	37.5	3	12.5	1			
Hanai 2004	single center in Japan	7	GMA	46	93.5	43	82.6	38	12 weeks	Rem= CAI≤4; Res= CAI had fallen, but still 4<	corticosteroids and/or 5-ASA/SAS
			steroid	23	78.3	18	65.2	15			
Hanai 2008	5 centers in Japan	11	GMA	35	80.0	28	74.3	26	12 weeks	Rem= CAI≤4; Res= CAI decreased by ≥5 points, but remained ≥5	all patients were on salicylates and the majority were on low dose PSL as well
			steroid	35	62.9	22	48.6	17			
Nakamura 2004	single center in Japan	5	GMA	10	N/A	N/A	80.0	8	6 weeks	based on CAI, but not specified	all patients received PSL; SAS and 5-ASA was unchanged
			no GMA	10	N/A	N/A	20.0	2			

Sands 2008 A study	13 centers in Japan, Austria Belgium, France, Germany, Italy, Norway, Sweden	10	GMA	31	67.7	21	16.1	5	12 weeks	Rem= Mayo score ≤2; 0-1 endoscopic score Res= Mayo score decrease ≥3,	one or more of the following: 5-ASA agents, PSN, 6-MP or AZA
			sham	16	62.5	10	18.8	3			
Sands 2008 B study	36 centers in the USA, Canada	10	GMA	112	60.7	68	17.0	19	12 weeks	Rem= Mayo score ≤2; 0-1 endoscopic score Res= Mayo score decrease ≥3	one or more of the following: 5-ASA, PSN, 6-MP or AZA
			sham	56	50.0	28	10.7	6			
Sawada 2005[4]	6 centers in Japan	7	GMA	10	80.0	8	20.0	2	10 weeks	Rem= CAI=0; Res= CAI improved >3	except for PSL, other medications remained unchanged
			sham	9	33.3	3	11.1	1			

Clinical remission maintenance

Study Name	Country/Setting	Number of cycles (n)	Randomization	Number of patients analyzed (n)	Number of patients in clinical remission at the end of the study		Close-out examination	Outcome criteria (Rem)	Concomitant medication
					%	n			
Emmrich 2006	single center in Germany	5	GMA	8	62.5	5	6 months	CAI≤4	all patients were on PSL; 5-ASA or SAS was allowed; AZA given at baseline remained unchanged
			no GMA	5	20.0	1			
Fukunaga 2012	single center in Japan	12	GMA	10	40.0	4	12 months	CAI≤4	stable dose of AZA and PSL were allowed if started before randomization
			sham	11	9.1	1			
Maiden 2008	3 centers in United Kingdom	5	GMA	18	77.8	14	6 months	CAI≤6	only 5-ASA or oral corticosteroid

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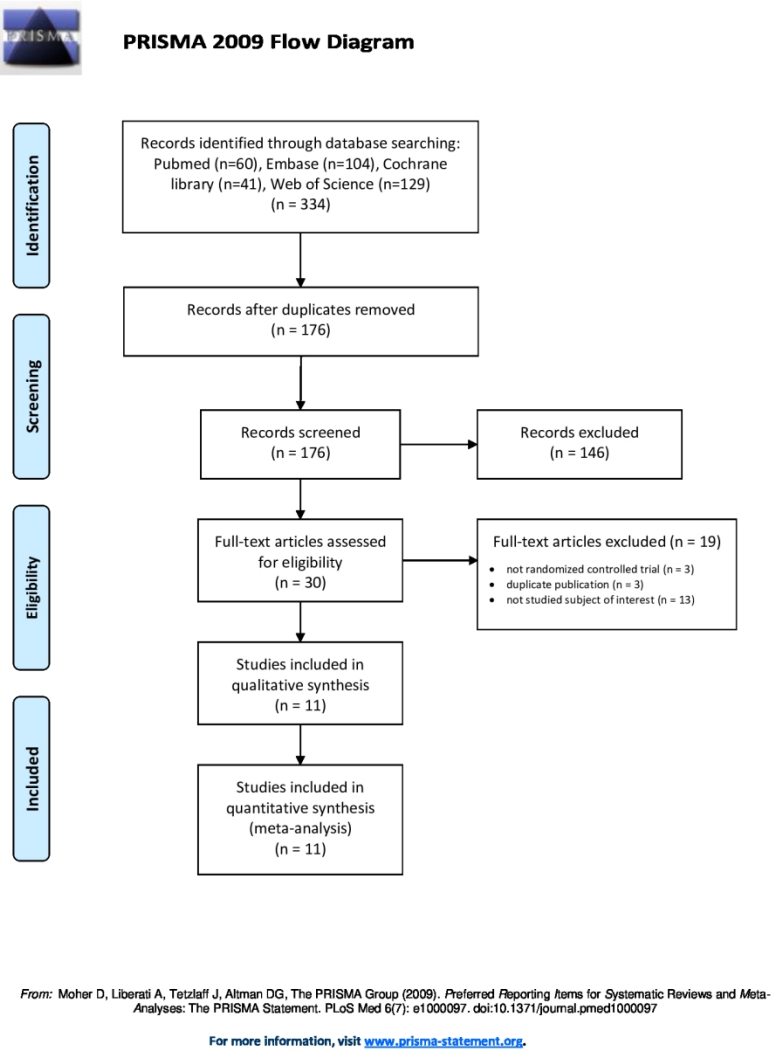

Figure 1: PRISMA flow chart representing the process of the study search and selection

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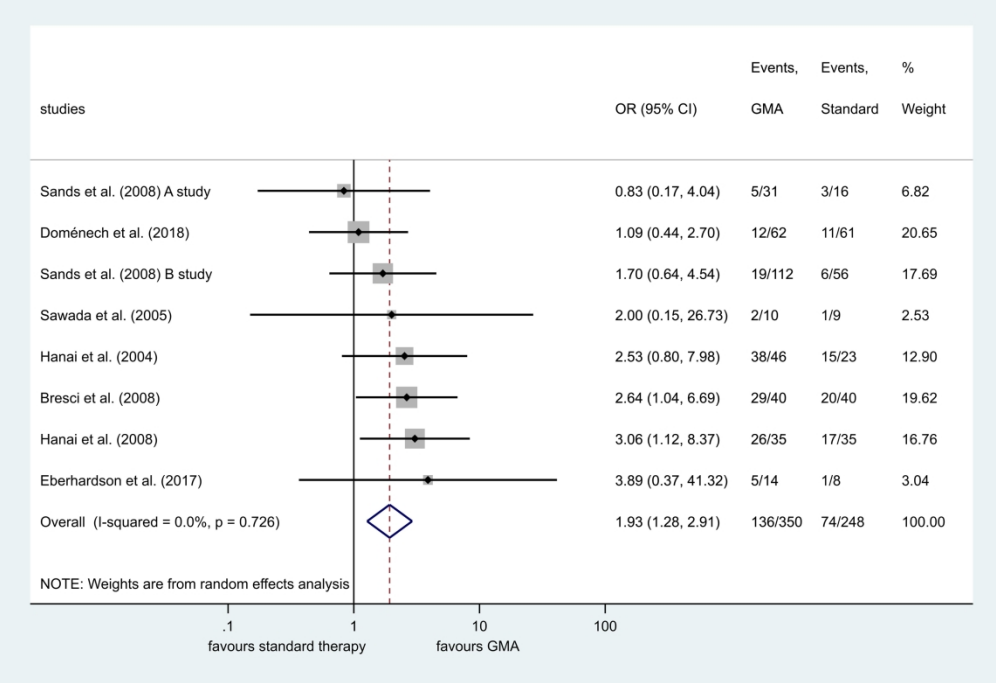

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

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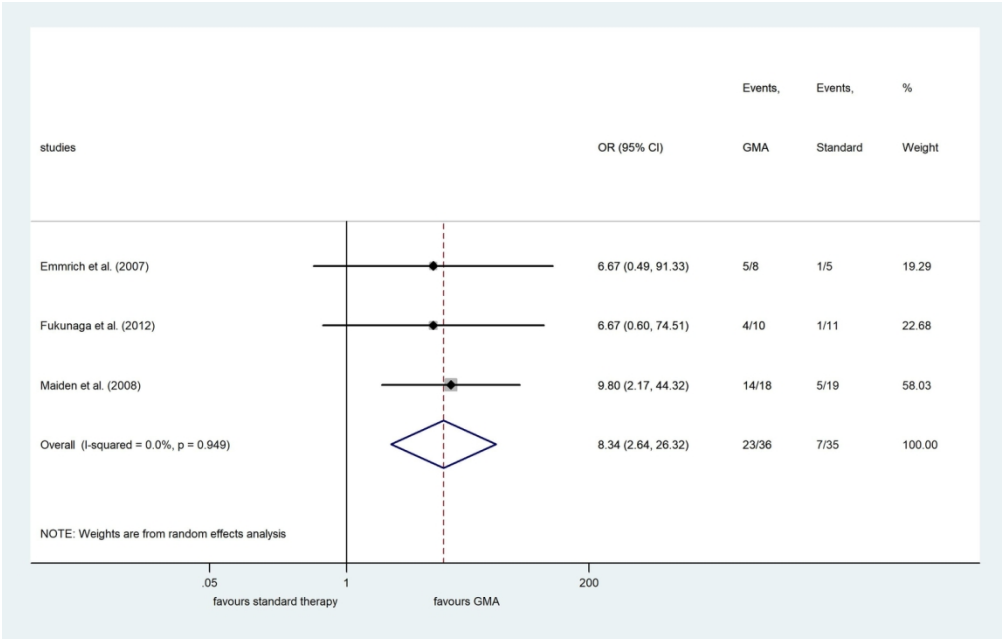

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

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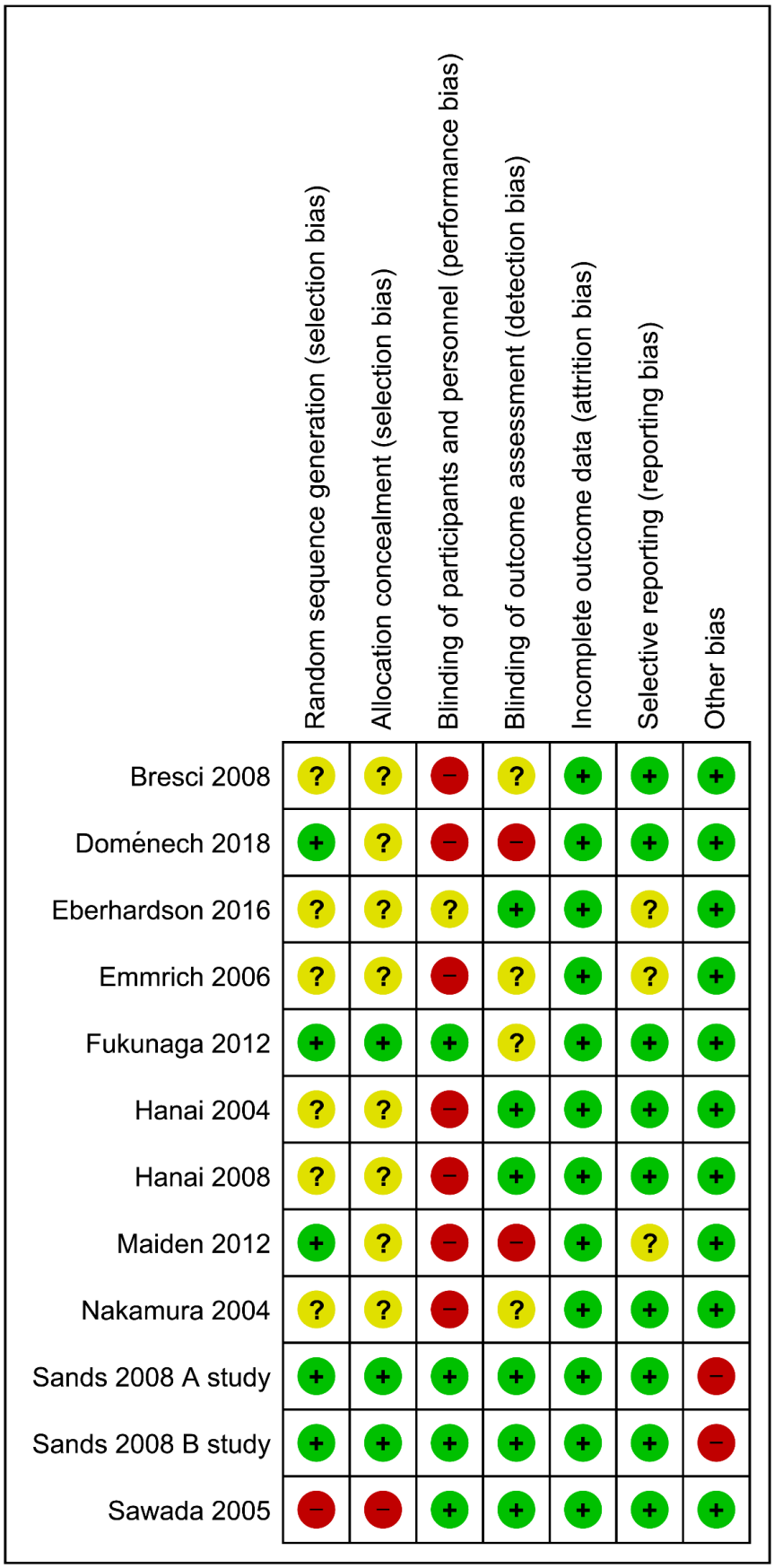
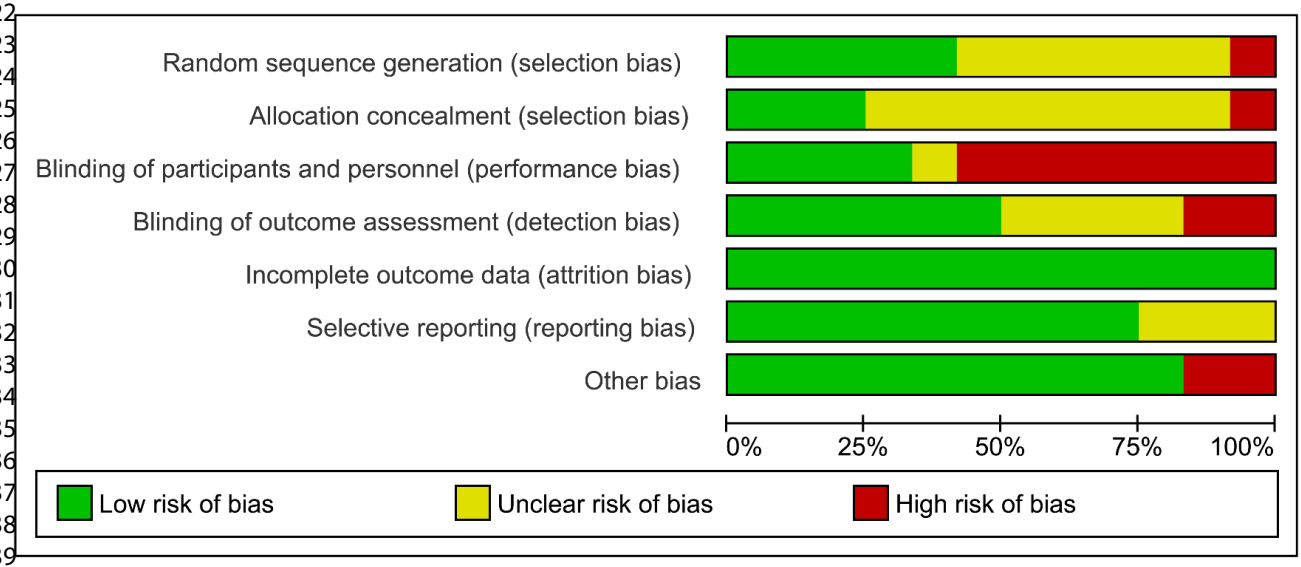
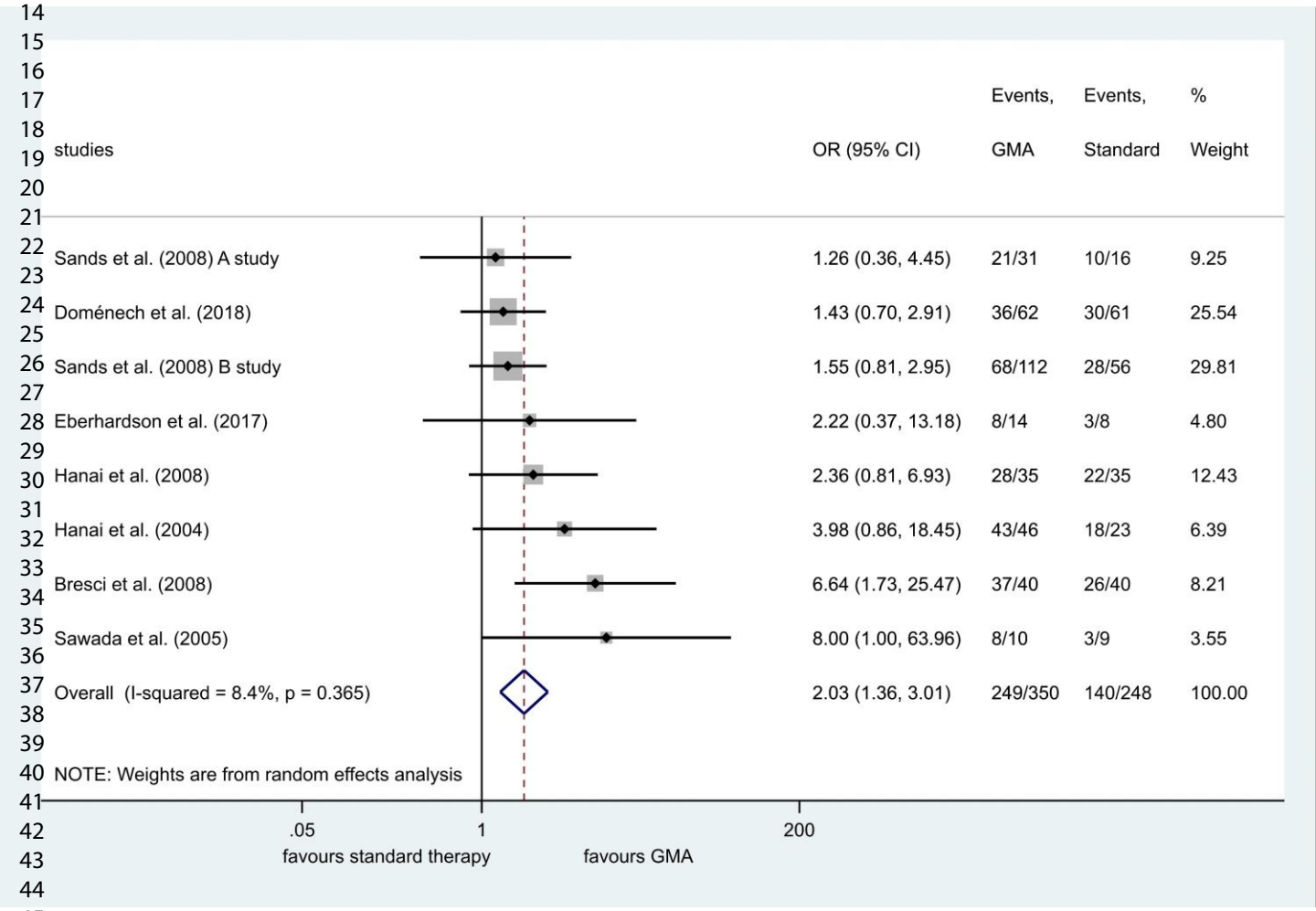
Supplementary Figure 1



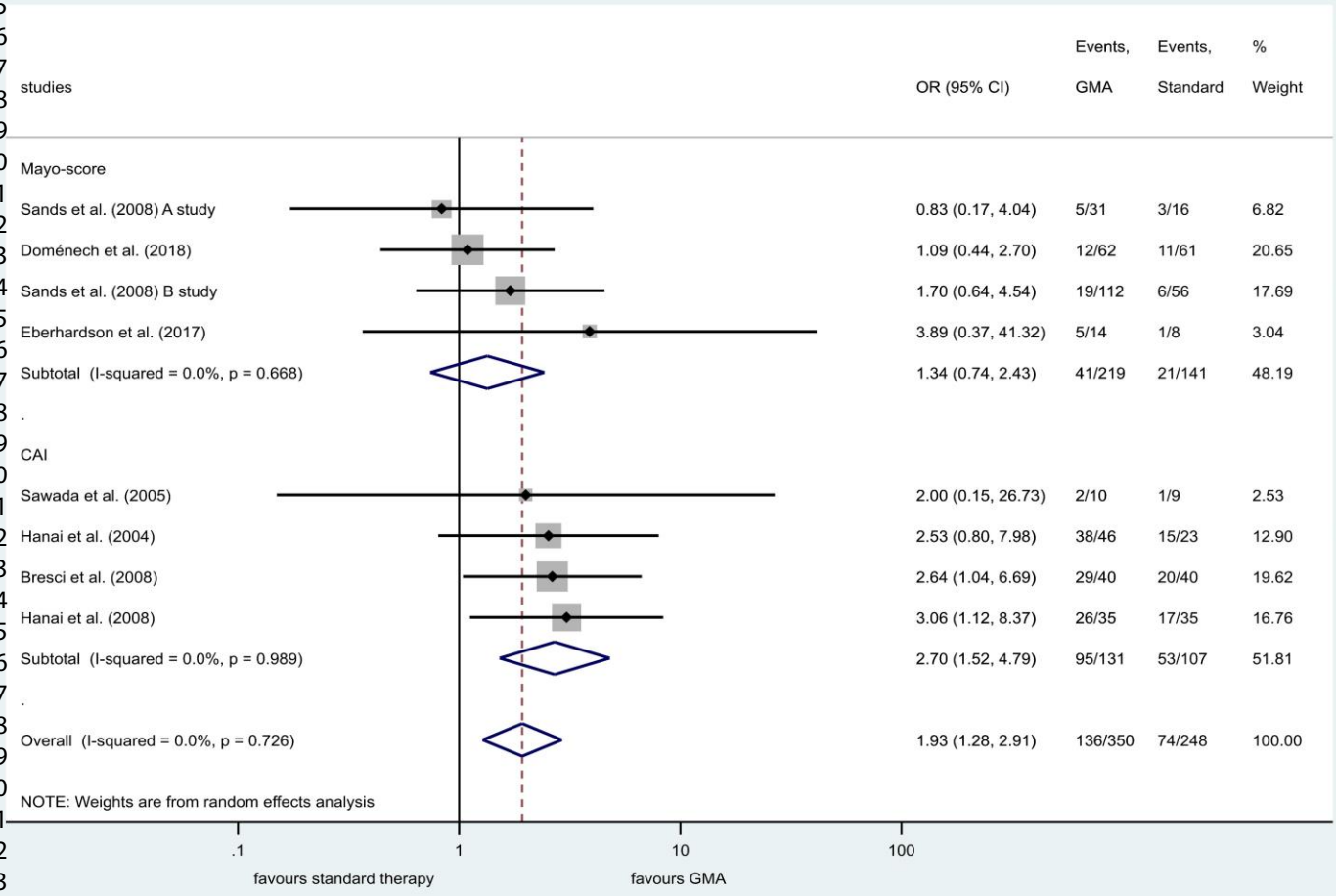
Supplementary Figure 2



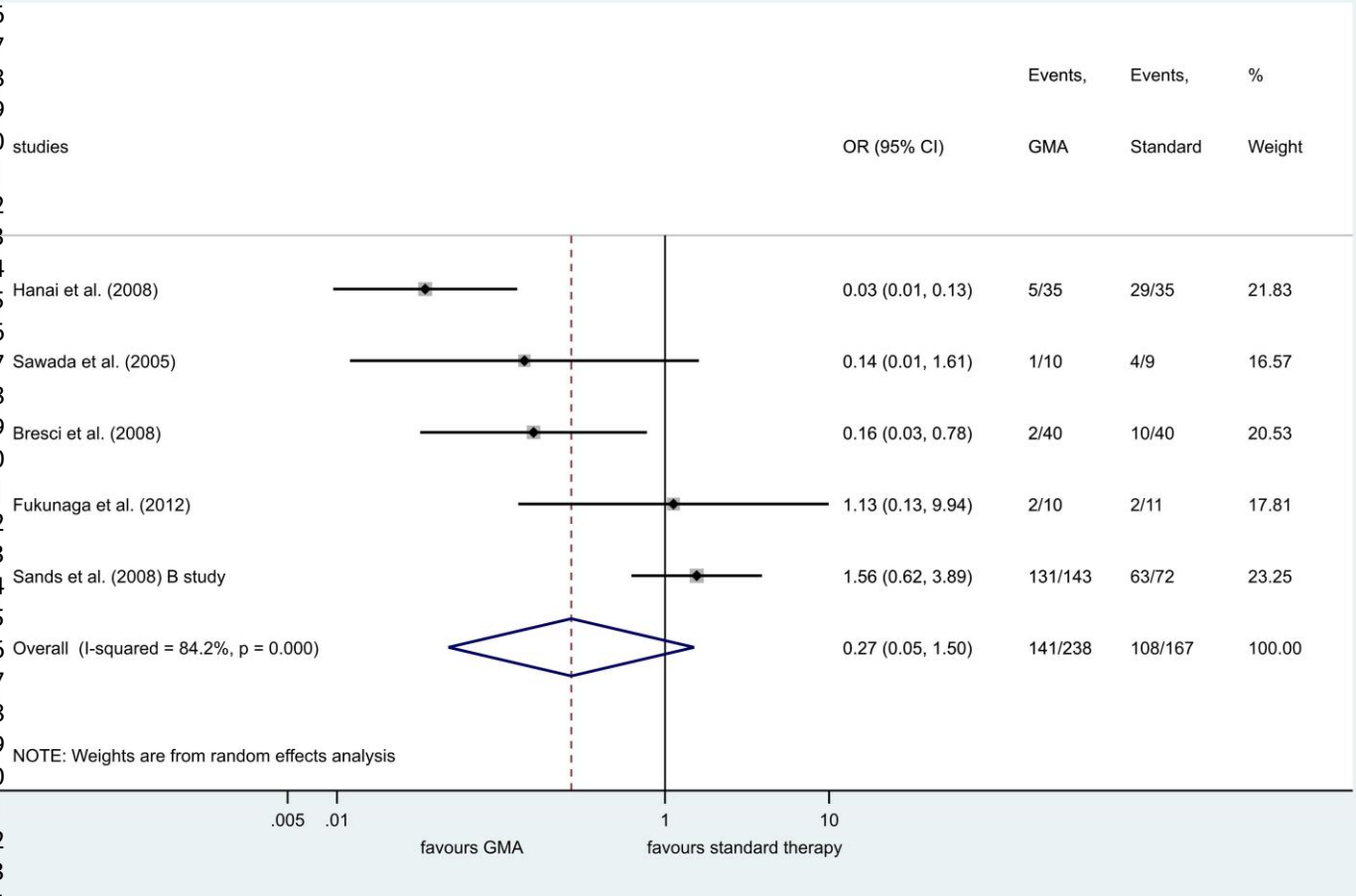
Supplementary Figure 3



Supplementary Figure 4

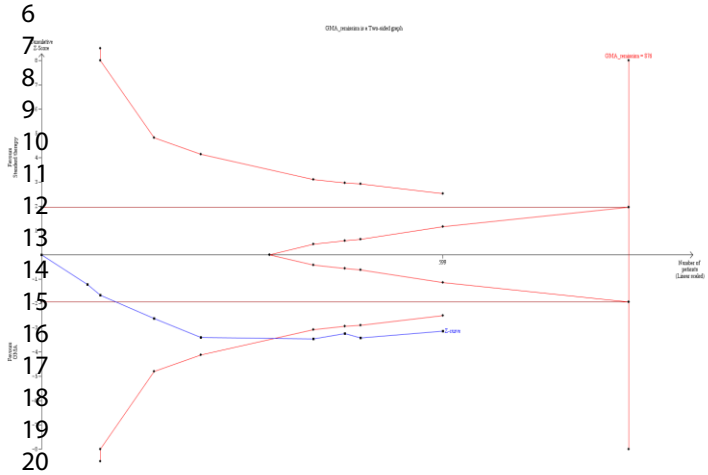


Supplementary Figure 5



Supplementary Figure 6

A



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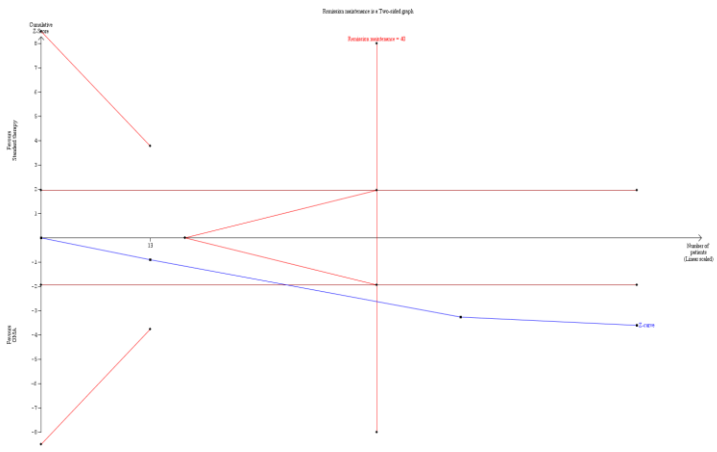

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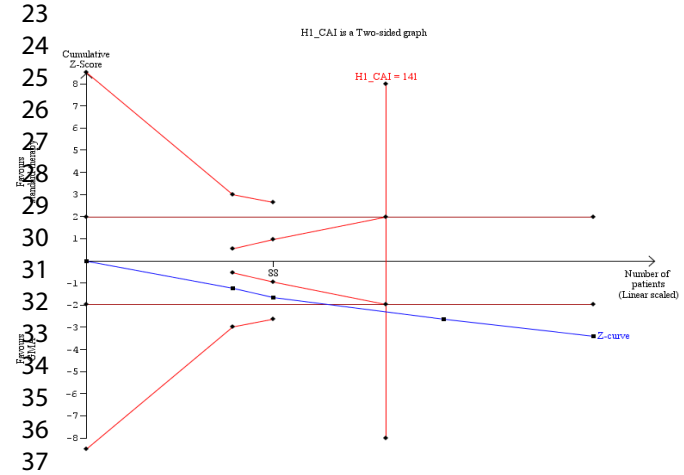

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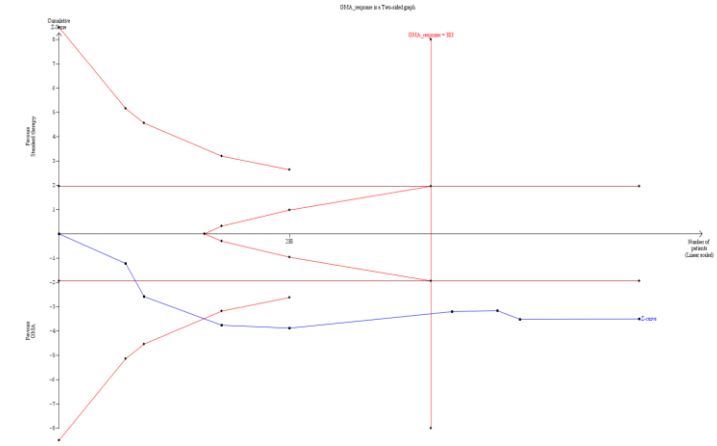

Supplementary Table 1

Hanai et al. 2004	flushing, nausea, mild fever
Sawada et al. 2005	fever, skin rash, back pain
Bresci et al. 2008	headache, gastrointestinal intolerance, facies lunaris, vascular hypertension, glucose intolerance
Fukunaga et al. 2012	nausea, skin itchiness
Sands et al. 2008	headache, disaese flare-up, decreased diastolic blood pressure, nasopharyngitis, hypotension, nausea, fatigue, post procedure hematoma, abdominal pain, dizziness, vomiting, vessel puncture site bruise, diarrhea, upper respiratory tract infection, flatulence

Supplementary Table 2

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	standard therapy for clinical remission induction and GMA as an adjunctive therapy	standard therapy for clinical remission induction	Relative (95% CI)	Absolute (95% CI)		
Clinical remission rate (assessed with: CAI or Mayo-score)												
1	randomised trials	serious	not serious	not serious	not serious	none	136/350 (38.9%)	74/249 (29.7%)	**OR 1.94** (1.28 to 2.92)	**153 more per 1 000** (from 54 more to 255 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Clinical response and clinical improvement (CAI or Mayo-score)												
1	randomised trials	serious	not serious	not serious	not serious	none	249/350 (71.1%)	140/249 (56.2%)	**OR 2.05** (1.37 to 3.06)	**162 more per 1 000** (from 75 more to 235 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Clinical remission maintenance rate (assessed with: CAI)												
1	randomised trials	serious	not serious	serious [a]	not serious	none	39/36 (108.3%)	17/35 (48.6%)	**OR 8.34** (2.64 to 26.32)	**402 more per 1 000** (from 228 more to 476 more)	⊕⊕◯◯ LOW	CRITICAL
Adverse events												
2	randomised trials	very serious	not serious	very serious [b]	very serious [c,d]	publication bias strongly suspected	141/238 (59.2%)	108/167 (64.7%)	**OR 0.27** (0.05 to 1.50)	**316 fewer per 1 000** (from 563 fewer to 86 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Steroid-sparing effect												
1	randomised trials	serious	not serious	not serious	very serious [d]	none	66	43	-	WMD **6.83 mg/day lower** (14.47 lower to 0.81 higher)	⊕◯◯◯ VERY LOW	IMPORTANT

CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Duration of follow-up differs among studies (6 months or 12 months). b. Pool of adverse events differs among studies. c. The optimal information size criterion is not met. d. TSA could not be carried out.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 3-4 Figure 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 4 Figure 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 4-5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Page 5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 4-5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 5 Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 5-6 Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 6 Suppl. Figure 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 6 Figure 2-3 Suppl. Figure 3-5
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency.	Page 6 Figure 2-3 Suppl. Figure 3-5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 6 Suppl. Figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 7 Suppl. Figure 4 Suppl. Figure 6
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 7&9 Suppl. Table 2
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 2&8

PRISMA 2009 Checklist

Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 9

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

BMJ Open

Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

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Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

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Keywords: Inflammatory bowel disease; IMMUNOLOGY; Gastroenterology; HAEMATOLOGY

1 Abstract

Objective: The goal of treatment in ulcerative colitis (UC) is to induce and maintain remission. The addition of granulocyte and monocyte apheresis (GMA) to conventional therapy may be a promising therapeutic alternative. In this meta-analysis, we aimed to assess the efficacy and safety profile of GMA as an adjunctive therapy.

Design: Systematic review and meta-analysis.

Methods: We searched four databases (MEDLINE, Embase, Web of Science, and Cochrane Central Register of Controlled Trials) for randomized or minimized controlled trials which discussed the impact of additional GMA therapy on clinical remission induction and clinical remission maintenance compared to conventional therapy alone. Primary outcome were clinical remission induction and maintenance, secondary outcomes were adverse events and steroid-sparing effect. Odds ratios (OR) with 95% confidence intervals were calculated. Trial Sequential Analyses (TSA) were performed to adjusts for the risk of random errors in meta-analyses.

Results: A total of eleven studies were eligible for meta-analysis. GMA was clearly demonstrated to induce and maintain clinical remission more effectively than conventional therapy alone (598 patients: OR: 1.93, CI: 1.28–2.91, p=0.002, I^2=0.0% for induction; 71 patients: OR: 8.34, CI: 2.64–26.32, p<0.001, I^2=0.0% for maintenance). There was no statistically significant difference in the number of adverse events (OR: 0.27, CI: 0.05–1.50, p=0.135, I^2=84.2%)

Conclusion: GMA appears to be more effective as an adjunctive treatment in inducing and maintaining remission in UC patients than conventional therapy alone.

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2 Article Summary

Strengths and limitations of this study

- This is the first meta-analysis assessing the role of GMA in clinical remission maintenance in ulcerative colitis.
- Grading of Recommendations Assessment, Development and Evaluation approach was applied to appraise the certainty of evidence.
- Our results are limited by the relatively low number of patients and the heterogenous reporting of adverse events.
- To address the limitation by the number of included patients and to control both type I and type II errors, Trial Sequential Analyses have been performed.

3 Introduction

Ulcerative colitis (UC) is one of two major types of inflammatory bowel disease (IBD). The incidence of this disease varies from nine to 20 cases per 100 000 person-years (1). UC is a lifelong illness that has a profound impact on patients. The primary goal of treatment is to achieve and maintain remission, thereby preventing colectomy and colorectal neoplasms and ensuring an acceptable quality of life (2). The choice of treatment for patients with UC is tied to the clinical and endoscopic severity of the disease along with the frequency and severity of relapses. Patients with no response to conventional therapies, especially to corticosteroids and immunosuppressive agents, are common candidates for biological treatments and/or surgery. However, both of these options are challenged by the high costs and incidence of side-effects and complications.

Patients with UC usually have a raised level of granulocytes, and, in the case of an active disease, the mucosa of the bowel is infiltrated by a large number of granulocytes and macrophages. These leukocytes release degradative enzymes and proinflammatory cytokines, which lead to further inflammation of the bowel. Based on the hypothesis that a reduction of activated granulocytes and monocytes/macrophages may be beneficial, granulocyte-monocyte apheresis (GMA) was proposed as a strategy to promote remission in active UC (3). GMA is a novel non-pharmacological treatment tool for patients with UC, comprising an extra-corporeal absorptive circuit, which decreases inflammatory cytokines and upregulates regulatory T cells. Despite its high cost, GMA seems to have a good safety profile (3).

However, data on the efficacy of GMA are still debated. The first studies published in Japan showed remission or response rates of up to 60–80% (4-6). Sands et al. reported a study with a large number of patients comparing GMA to a placebo, and they found no significant difference in terms of clinical response (7). This substantial difference between studies could be explained by the heterogeneity of patients' characteristics, most probably by the varying severity and extent of the disease.

A large proportion of patients require long-term, high-dose steroid treatment, which often results in severe side-effects impairing patients' quality of life. If addition of GMA can reduce the dose of corticosteroids, the risk of steroid-induced adverse events (AEs) could be minimized. Therefore, it is also essential to evaluate the steroid-sparing effects of GMA (8). Beyond the induction of remission and the impact on steroid requirement, the role of GMA in maintaining remission is unclear (9). The aim of our study was to assess the role of GMA in the induction and maintenance of clinical remission in UC and to evaluate the potential steroid-sparing effect of the therapy.

4 Methods

The meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (10). The review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews (CRD42019134050).

4.1 Search strategy

The systematic literature search was conducted by two independent reviewers (KS and FM) in MEDLINE (via PubMed), EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science for studies published up to 5th March 2019. The search query in each database was based on PICO components combined with Boolean operators: (gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND ("inflammatory

bowel disease" OR "ulcerative colitis") AND (random*). Details of our search strategy and terms are presented in supplementary material.

4.2 Eligibility criteria

General criteria: a randomized controlled trial (RCT) or minimized controlled trial (This type of sequence generation is considered to be nearly equivalent to being random) (11); only full-text articles were included.

Specific criteria for clinical remission induction: patients with active UC ($Population_1$), standard therapy for remission induction and GMA as an adjunctive therapy ($Intervention_1$), and standard therapy for remission induction ($Comparison_1$); $Outcomes_1$: clinical response rate and clinical remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.

Specific criteria for clinical remission maintenance: patients with UC in clinical remission induced by GMA ($Population_2$), standard therapy for remission maintenance and GMA as an adjunctive therapy ($Intervention_2$), and standard therapy for remission maintenance ($Comparison_2$); $Outcomes_2$: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

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.

The titles of the studies were screened based on predefined criteria, and the relevant studies were selected for abstract review. If the abstract was found to be appropriate, the full text of the article was studied. The decision to include a study in the meta-analysis was based on an independent assessment by the two reviewers and eventually by consensus for resolution of any disagreements. Reference lists in included studies and reviews on this topic were searched for additional studies. Publications citing the included studies were also screened in the Google Scholar academic search engine.

4.3 Data extraction

The two investigators (KS and FM) reviewed the articles independently and extracted data into a standardized data collection form (discrepancies were resolved based on consensus). For the selected studies, characteristics were extracted, including publication year, country, number of centres, number of patients, and study design. In addition, patient characteristics (age, sex, and extent of disease), details of therapy (concomitant medication, volume of GMA, number of GMA cycles, and duration of treatment), and main outcomes (number of patients with clinical improvement/response, number of patients achieving clinical remission, number of patients with maintained remission, and number of AEs) were also extracted.

4.4 Risk of bias assessment

The Cochrane Risk of Bias Tool was used by the two independent investigators (KS and FM) to assess the quality of the studies included. Any disagreement was resolved based on consensus (12). Major domains of quality assessment were the following:

1. Random sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)

5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)
7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials, and imputation of intention-to-treat (ITT) analysis)

4.5 Statistical analysis

The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR) with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered statistically significant (except for heterogeneity, for which a p value <0.10 was considered significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR, WMD, and weights are presented in forest plots. The random-effects model was used to pool effect sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating Higgins' I^2 indicator (13, 14). The Q statistics were computed as the squared deviations from the pooled effect of the weighted sum of individual study effects, with the weights being used in the pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees of freedom (where k was the number of studies). The I^2 index corresponds to the percentage of the total variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–60%), substantial (50–90%), and considerable (75–100%) (11). Subgroup analysis was performed as described in the study protocol if a sufficient number of studies was available. Funnel plots were used to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal information size (OIS). This methodology combines an information size with the threshold of statistical significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).

4.6 Quality of evidence

The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality of evidence for each outcome (15, 16). Disagreements were resolved by consensus.

5 Results

5.1 Search and selection

The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After screening and assessment for eligibility, eleven full-text articles containing one minimized controlled trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active UC, and three studies contained data on patients with UC in clinical remission.

5.2 Characteristics of the studies included

The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients received GMA, and 248 were in control groups. All the participants had active UC and were treated with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received standard of care added to the intervention/comparator and they did not receive any anti-TNF agent.

Both GMA and control were added to conventional treatment. In terms of main outcomes, the studies investigated the rate of clinical remission and clinical response. Investigators assessed the activity of UC with either the Mayo score or CAI. One study required steroid-free remission to regard cases as being in clinical remission.

In the case of clinical remission maintenance, all the studies were randomized controlled trials. A total of 71 participants (mean: 24, ranging from 13 to 37) were included in this meta-analysis: 36 patients received GMA, and 35 were in control groups. All the participants had ulcerative colitis in remission and were treated with Adacolumn® or Cellsorba®. One trial evaluated GMA vs sham control (24) and two trials assessed GMA compared to standard therapy alone (9, 25). Both GMA and sham control were added to conventional treatment. In terms of main outcome, the studies investigated the rate of clinical relapse.

Three studies also reported on the steroid-sparing effect of GMA (9, 17, 22).

5.3 Risk of bias assessment

A summary of risk of bias assessment is shown in **Supplementary Figure 1 and Supplementary Figure 2**. Three unblinded studies were at high risk of performance bias (19, 22, 25). Because of the nature of the intervention, four studies which lacked a description of the blinding process were interpreted as having a high risk of bias (18, 21, 23, 24). As regards assessment blinding, two unblinded studies were judged to be at high risk of bias (19, 25). Two studies were deemed as having a high risk of other bias; although they used ITT analysis, they considered subjects who left the study as a treatment failure that may lead to bias (7).

5.4 Efficacy and safety of GMA in clinical remission induction

Seven randomized and one minimized controlled trial evaluated clinical remission induction. GMA therapy was associated with a better clinical response rate compared to the control group (OR = 2.03, 95% CI = 1.36–3.01, p<0.001, I^2 = 8.4%) (**Supplementary Figure 3**). Subgroup analysis of studies with assessment at 12 weeks also showed benefit (OR = 1.67, 95% CI = 1.12–2.49, p=0.012, I^2 = 0.0%) **(Supplementary Figure 4)**. Patients undergoing GMA therapy had a higher remission rate compared to standard therapy without GMA (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I^2 = 0.0%) (**Figure 2**). Sub-group analyses were performed based on activity indices and number of GMA cycles. No difference was found between the two groups in studies assessing UC with the Mayo score (OR = 1.34, 95% CI = 0.74–2.43, p=0.334, I^2 = 0.0%), but the remission induction was more successful in studies using CAI for assessment (OR = 2.70, 95% CI = 1.52–4.79, p=0.001, I^2 = 0.0%) (**Supplementary Figure 5**). A significant difference was found in studies using five cycles compared to the control (OR = 2.78, 95% CI = 1.17–6.60, p=0.021, I^2 = 0.0%) and more than five cycles compared to standard therapy alone (OR = 1.73, 95% CI = 1.08–2.77, p=0.022, I^2 = 0.0%). There was no statistically significant difference in the number of AEs (p=0.135) (**Supplementary Figure 6**). No statistically significant steroid-sparing effect was detected among patients with active UC (p=0.080). A list of reported AEs is presented in **Supplementary Table 1**.

5.5 Efficacy and safety of GMA in clinical remission maintenance

Three randomized clinical trials evaluated the clinical remission rate in remitting UC induced by GMA. Patients receiving GMA had a higher rate of clinical remission maintenance (OR = 8.34, 95% CI = 2.64–26.32, p<0.001, I^2 = 0.0%) (**Figure 3**). Due to lack of data, the rate of AEs could not be assessed in this population.

5.6 Trial Sequential Analysis

Based on a TSA, the cumulative Z curve crossed the trial sequential significance boundary as regards clinical remission induction and clinical remission maintenance (power=80.0%; alpha=5.0%) (**Supplementary Figure 7**). Moreover, clinical remission maintenance exceeded the required meta-analysis sample size, possibly suggesting that further clinical trials are not required. A TSA for AEs and steroid-sparing effects could not be carried out due to insufficient information size.

5.7 Quality of evidence

The GRADE analysis rated the quality of evidence for primary and secondary outcomes at a very low to low level. GRADE evidence profile is shown in **Supplementary Table 2**.

6 Discussion

The main goal of care is to achieve and maintain remission of UC. This condition is usually treated by a step-up approach, during which treatments are switched or additional treatment is administered to optimize current therapy. There are several therapeutic agents to slow down the clinical activity of UC. Corticosteroids, 5-aminosalicylates, immunosuppressive agents, and tumour necrosis alpha-inhibitors are commonly used, and new therapeutic targets, such as anti-adhesion molecules and anti-interleukins, are emerging. Despite these multiple therapeutic options, there is still a need to expand the scope of treatment methods due to possible development of intolerance or resistance to current treatments. After running out of treatment options, surgical therapy is frequently the last remaining option for patients. GMA is a novel non-pharmacologic treatment option for active and remitting UC, by which activated granulocytes and monocytes are removed from the circulation. These cells may contribute to the pathogenesis of UC.

Guidelines describing the role of GMA in UC are in agreement on the potential beneficial effect and favourable safety profile. They also agree that there is insufficient evidence in this field of practice (26, 27).

To our knowledge, the first report on the efficacy of GMA in UC was published in Japan in 2001 (28). This study found a considerably high remission rate with only five sessions of GMA in patients refractory to conventional drug therapy. Subsequent studies from the early 21st century had similar results (29-31). In 2008, Sands et al. failed to prove a significant difference in clinical remission rate between GMA and a placebo on a relatively large population (7). However, this study was not free of attrition bias; a high proportion of patients were lost to follow-up. Three systematic reviews and meta-analyses have been conducted in this field so far (32-34). All of them have agreed on the benefit of GMA in clinical remission induction, and they pointed out the necessity for more trials with a rigorous and clear design to further narrow the focus on specific patient groups. These studies used one to three databases for a systematic search and selection.

In our current meta-analysis, a broader literature search was carried out, and the role of GMA in clinical remission maintenance was assessed. Our work supported the hypothesis that GMA improves the rates of clinical response and clinical remission in patients with UC. It should be noted that response and remission rates defined by symptom scores should be cautiously interpreted because they also include subjective elements, such as overall physician judgement on disease activity. A few recent retrospective and prospective studies have suggested certain prognostic factors in the therapeutic response (35-37). It seems that younger patients respond better to GMA therapy, whereas gender and smoking status showed no difference in response to treatment (35). Yokoyama et al. found that shorter duration of UC and lower cumulative corticosteroid dose are associated with a higher efficacy rate

(36). In their study, patients who received GMA treatment immediately after relapse were the best responders. It would be advisable to conduct further research to identify subgroups of UC where patients benefit the most from GMA (38).

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%. To date, there is no uniformly accepted GMA regimen. There are RCTs to compare a ten-cycle and a five-cycle GMA regimen. Dignass et al. and Ricart et al. found similar remission rates between ten and five cycles (46% vs. 36%, p=0.479; 35.7% vs 45.5%, p>0.05, respectively) (38, 39). The latter study also showed a steroid-sparing effect in the group receiving ten cycles of GMA. Sakuraba et al. found that an improved remission rate is associated with intensive GMA (54.0% vs 71.2%, p=0.029 in five- and ten-cycle regimens, respectively) (40). In our meta-analysis, the number of GMA cycles varied among studies as well. We assessed the efficacy of GMA based on the two main regimens in previous trials. Both groups showed a benefit of adding GMA to the therapy compared to standard treatment alone.

Regarding the induction and maintenance of remission, our results relate to clinical remission. In 2015, based on insights from various clinical trials, a new consensus was made on appropriate evidence-based treatment targets (41). From then on, in addition to controlling symptoms, more objective markers came to the fore and endoscopic remission came to the spotlight. Only three of the articles analysed reported a comparison of endoscopic remission. Nakamura et al. found that the improvement in endoscopic score was significantly higher in the group receiving GMA as well (23). Another study showed that the Rachmilewitz's endoscopic index was significantly improved in patients treated with GMA compared to the control group (17). The third study reported similar endoscopic remission rate in the two groups (12% vs 11% in GMA and sham group, respectively; p=1.00) (7). Data on objective inflammatory markers are also contradictory and insufficient (18, 20, 25). In light of this, there is a need for additional, high-quality RCTs that focus on current therapeutic targets.

We found no significant difference between the two groups as regards AEs (very low certainty). Further studies are called for to provide a higher level of evidence on this topic. They would be particularly important for specific subgroups where the safety profile is of paramount importance, such as in cytomegalovirus infection, adolescence, and pregnancy. Clinical trials should also target these populations because fewer therapeutic options are available for them and the safety profile of GMA seems favourable compared to other treatments.

As with any therapeutic option, cost-effectiveness should also be considered. The cost of GMA is much higher compared to regular medication, such as corticosteroids, but GMA could be cost-effective in the long term. The use of GMA may reduce the cost of medical services, hospitalization, and surgery in the long term. Nevertheless, GMA's safety profile is in sharp contrast to multiple severe AEs associated with conventional pharmacologics and biologics. According to recommendations, if UC flares up, treatment is usually escalated to biologics. As GMA and biologics are also likely to differ in terms of invasiveness, safety, and efficacy, the question arises: which one may be more beneficial? However, there is currently no evidence of this. In this regard, limited data are available from recent studies suggesting that GMA may be beneficial in patients who no longer respond to biologics (42-44).

To our knowledge, this is the first meta-analysis to assess the role of GMA in UC remission maintenance. Our study showed that the addition of GMA enhances the proportion of patients who can maintain their remission (low certainty). Fukunaga et al. and Emmrich et al. enrolled clinically active UC patients based on CAI (9, 24). After successful induction therapy with the inclusion of GMA,

patients achieving clinical remission were allocated to groups with and without GMA treatment for remission maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin, which is considered as a risk factor of relapse (25). Their results showed that faecal calprotectin level significantly decreases following five treatment session. This study differs from the previous two in the fact that they enrolled an asymptomatic population regardless of how patients achieved remission. The two studies recruiting patients with active UC detected no statistically significant difference between study arms in time to first relapse; however, it must be noted that in one of these studies, all the patients became steroid-free in the GMA group (9). 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). Despite our very promising results, these findings are limited by the amount of available data. More randomized controlled trials are necessary in this area to strengthen our results. This study has some potential limitations. 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**). Moreover, our funnel plots showed symmetry by visual assessment, but publication bias still cannot be ruled out because of the low number of included studies. Side-effects and safety data were not uniformly reported in most of the publications under analysis, according to the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines (15). Therefore, our second main objective, the safety assessment of GMA, was only achieved to a limited extent. Furthermore, this result is strongly limited by the high heterogeneity of studies. The most likely source of this is the heterogeneous nature of concomitant treatment. All in all, GMA seems to be a reasonable therapeutic option, but finding its exact place to treat UC demands further research. A particularly promising area could be remission maintenance.

6.1 Conclusion

Implications for practice: The results support the hypothesis that patients with active UC have a better chance of clinical remission if GMA is administered as an adjunctive therapy. As regards the frequency of AEs, we found no statistically significant difference between the two groups. With regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

Implications for research: Further studies are required to select patients who may benefit the most from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its role in remission induction. There is currently evidence available about induction and maintenance of clinical remission; however, the role of GMA concerning endoscopic and histological remission is currently unclear. If GMA is proven to be safe and effective, cost-effectiveness studies will also be worthwhile in the future.

7 Data availability statement

The data that support the findings of this study are available from the corresponding author, [A.H.], upon reasonable request.

8 Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

9 Author contributions

All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

S.K.: drafting the manuscript, selection of studies, data extraction, risk of bias assessment; D.N.: statistical analysis, preparation of the standardized data collection sheet, drafting the manuscript; P.H.: substantial contribution in study design, critical revision of the content; M.F.: selection of studies, data extraction, risk of bias assessment, drafting the manuscript; Z.S.: participation in the design of the study and its coordination, critical revision of the manuscript; B.E.: provided revisions to the scientific content of the manuscript, substantial contribution in design of the work; B.T.: substantial contribution in study design, drafting the manuscript; P.J.H.: preparation of the standardized data collection sheet, stylistic and grammatical revision of the manuscript, substantial contribution in study design; P.S.: expert in the field of gastroenterology, substantial contribution in study design and interpretation of data, preparation of study protocol and the first draft of the manuscript; A.H.: expert in the field of haematology, substantial contribution in study design and interpretation of data, preparation of study protocol and the first draft of the manuscript

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11 Conflict of interest

Authors do not have any conflicts of interest to declare.

12 Compliance with Ethical Standards

This study was prepared in accordance with the Committee on Publication Ethics (COPE) guidelines to respect third parties rights such as copyright and/or moral rights. Ethical approval was not required to conduct this project as data is not individualized and primary data was not collected.

13 Abbreviations

AE, adverse events; clinical activity index, CAI; confidence interval, CI; granulocyte and monocyte apheresis, GMA; inflammatory bowel disease, IBD; OR, odds ratio; RCT, randomized controlled trial; TSA, trial sequential analysis; UC, ulcerative colitis; weighted mean difference, WMD.

14 References

1. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. The Lancet. 2012;380(9853):1606-19.

2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis. The Lancet. 2017;389(10080):1756-70.

3. Saniabadi AR, Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H. Treating inflammatory bowel disease by adsorptive leucocytapheresis: a desire to treat without drugs. World J Gastroenterol. 2014;20(29):9699-715.

4. Matsumoto T, Fukunaga K, Kamikozuru K, Tozawa K, Yokoyama Y, Kusaka T, et al. Cytapheresis as a Non-Pharmacological Therapy for Inflammatory Bowel Disease. Transfus Med Hemother. 2008;35(1):18-23.

5. Yamamoto T. The role of granulocyte and monocyte apheresis in inflammatory bowel disease. J Crohns Colitis. 2013;7(3):e114.

6. Yamamoto T, Umegae S, Matsumoto K. Long-term clinical impact of early introduction of granulocyte and monocyte adsorptive apheresis in new onset, moderately active, extensive ulcerative colitis. J Crohns Colitis. 2012;6(7):750-5.

7. Sands BE, Sandborn WJ, Feagan B, Lofberg R, Hibi T, Wang T, et al. A randomized, double-blind, sham-controlled study of granulocyte/monocyte apheresis for active ulcerative colitis. Gastroenterology. 2008;135(2):400-9.

8. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Treatment of patients with acute ulcerative colitis: conventional corticosteroid therapy (MP) versus granulocytapheresis (GMA): a pilot study. Dig Liver Dis. 2007;39(5):430-4.

9. Fukunaga K, Yokoyama Y, Kamokozuru K, Nagase K, Nakamura S, Miwa H, et al. Adsorptive granulocyte/monocyte apheresis for the maintenance of remission in patients with ulcerative colitis: a prospective randomized, double blind, sham-controlled clinical trial. Gut Liver. 2012;6(4):427-33.

10. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

11. Hoboken. Cochrane Handbook for Systematic Reviews of Interventions: Wiley; 2008.

12. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

13. Cochran WG. The Combination of Estimates from Different Experiment. Biometrics. 1954;10:101-29.

14. Julian P T Higgins SGT, Jonathan J Deeks, Douglas G Altman. Measuring inconsistency in meta-analyses. British Medical Journal. 2003;327:557-60.

15. Bhatt A. International Council for Harmonisation E6(R2) addendum: Challenges of implementation. Perspectives in Clinical Research. 2017;8(4):162-6.

16. Schünemann H, Brożek J, Guyatt G, A O. GRADE handbook for grading quality of evidence and strength of recommendations: The GRADE Working Group, 2013; 2013.

17. Sawada K, Kusugami K, Suzuki Y, Bamba T, Munakata A, Hibi T, et al. Leukocytapheresis in ulcerative colitis: results of a multicenter double-blind prospective case-control study with sham apheresis as placebo treatment. Am J Gastroenterol. 2005;100(6):1362-9.

18. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Granulocytapheresis versus methylprednisolone in patients with acute ulcerative colitis: 12-month follow up. J Gastroenterol Hepatol. 2008;23(11):1678-82.

19. Domenech E, Panes J, Hinojosa J, Annese V, Magro F, Sturniolo GC, et al. Addition of Granulocyte/Monocyte Apheresis to Oral Prednisone for Steroid-dependent Ulcerative Colitis: A Randomized Multicentre Clinical Trial. J Crohns Colitis. 2018;12(6):687-94.

20. Eberhardson M, Karlen P, Linton L, Jones P, Lindberg A, Kostalla MJ, et al. Randomised, Double-blind, Placebo-controlled Trial of CCR9-targeted Leukapheresis Treatment of Ulcerative Colitis Patients. J Crohns Colitis. 2017;11(5):534-42.

21. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Kageoka M, et al. Intensive granulocyte and monocyte adsorption versus intravenous prednisolone in patients with severe ulcerative colitis: an unblinded randomised multi-centre controlled study. Dig Liver Dis. 2008;40(6):433-40.

22. Hanai H, Watanabe F, Yamada M, Sato Y, Takeuchi K, Iida T, et al. Adsorptive granulocyte and monocyte apheresis versus prednisolone in patients with corticosteroid-dependent moderately severe ulcerative colitis. Digestion. 2004;70(1):36-44.

23. Nakamura T, Kawagoe Y, Matsuda T, Ueda A, Ueda Y, Takahashi Y, et al. Effect of granulocyte and monocyte adsorption apheresis on urinary albumin excretion and plasma endothelin-1 concentration in patients with active ulcerative colitis. Blood Purif. 2004;22(6):499-504.

24. Emmrich J, Petermann S, Nowak D, Beutner I, Brock P, Klingel R, et al. Leukocytapheresis (LCAP) in the management of chronic active ulcerative colitis--results of a randomized pilot trial. Dig Dis Sci. 2007;52(9):2044-53.

25. Maiden L, Takeuchi K, Baur R, Bjarnason I, O'Donohue J, Forgacs I, et al. Selective white cell apheresis reduces relapse rates in patients with IBD at significant risk of clinical relapse. Inflamm Bowel Dis. 2008;14(10):1413-8.

26. Leukapheresis for inflammatory bowel disease: Interventional procedures guidance 2005 [Available from: https://www.nice.org.uk/guidance/ipg126.

27. Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, et al. Evidence-based clinical practice guidelines for inflammatory bowel disease. J Gastroenterol. 2018;53(3):305-53.

28. Shimoyama T, Kimura K, Hiwatashi N, Matsueda K, Munakata A, Asakura H, et al. Safety and Efficacy of Granulocyte and Monocyte Adsorption Apheresis in Patients With Active Ulcerative Colitis: A Multicenter Study. Journal of Clinical Apheresis. 2001;16:1-9.

29. Kanke K, Nakano M, Hiraishi H, Terano A. Clinical evaluation of granulocyte/monocyte apheresis therapy for active ulcerative colitis. Dig Liver Dis. 2004;36(12):811-7.

30. Kim HJ, Kim JS, Han DS, Yang SK, Hahm KB, Lee WI, et al. [Granulocyte and monocyte adsorption apheresis in Korean conventional treatment-refractory patients with active ulcerative colitis: a prospective open-label multicenter study]. The Korean journal of gastroenterology = Taehan Sohwagi Hakhoe chi. 2005;45(1):34-44.

31. Shimoyama T, Sawada K, Hiwatashi N, Sawada T, Matsueda K, Munakata A, et al. Safety and efficacy of granulocyte and monocyte adsorption apheresis in patients with active ulcerative colitis: a multicenter study. J Clin Apher. 2001;16(1):1-9.

32. Habermalz B, Sauerland S. Clinical effectiveness of selective granulocyte, monocyte adsorptive apheresis with the Adacolumn device in ulcerative colitis. Dig Dis Sci. 2010;55(5):1421-8.

33. Thanaraj S, Hamlin PJ, Ford AC. Systematic review: granulocyte/monocyte adsorptive apheresis for ulcerative colitis. Aliment Pharmacol Ther. 2010;32(11-12):1297-306.

34. Yoshino T, Nakase H, Minami N, Yamada S, Matsuura M, Yazumi S, et al. Efficacy and safety of granulocyte and monocyte adsorption apheresis for ulcerative colitis: a meta-analysis. Dig Liver Dis. 2014;46(3):219-26.

35. Imperiali G, Amato A, Terpin MM, Beverina I, Bortoli A, Devani M, et al. Granulocyte-Monocyte Apheresis in Steroid-Dependent, Azathioprine-Intolerant/Resistant Moderate Ulcerative Colitis: A Prospective Multicenter Study. Gastroenterol Res Pract. 2017;2017:9728324.

36. Yokoyama Y, Kawai M, Fukunaga K, Kamikozuru K, Nagase K, Nogami K, et al. Looking for predictive factors of clinical response to adsorptive granulocyte and monocyte apheresis in patients with ulcerative colitis: markers of response to GMA. BMC Gastroenterology. 2013;13(27).

37. Tanaka T, Sugiyama S, Goishi H, Kajihara T, Akagi M, Miura T. Treatment of children and adolescents with ulcerative colitis by adsorptive depletion of myeloid lineage leucocytes as monotherapy or in combination with low dose prednisolone after failure of first-line medications. BMC Gastroenterology. 2013;13(130).

38. Dignass AU, Eriksson A, Kilander A, Pukitis A, Rhodes JM, Vavricka S. Clinical trial: five or ten cycles of granulocyte-monocyte apheresis show equivalent efficacy and safety in ulcerative colitis. Aliment Pharmacol Ther. 2010;31(12):1286-95.

39. Ricart E, Esteve M, Andreu M, Casellas F, Monfort D, Sans M, et al. Evaluation of 5 versus 10 granulocyteaphaeresis sessions in steroid-dependent ulcerative colitis: A pilot, prospective, multicenter, randomized study. World Journal of Gastroenterology. 2007;13(15):2193-7.

40. Sakuraba A, Motoya S, Watanabe K, Nishishita M, Kanke K, Matsui T, et al. An open-label prospective randomized multicenter study shows very rapid remission of ulcerative colitis by intensive granulocyte and monocyte adsorptive apheresis as compared with routine weekly treatment. Am J Gastroenterol. 2009;104(12):2990-5.

41. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol. 2015;110(9):1324-38.

42. Yokoyama Y, Sawada K, Aoyama N, Yoshimura N, Sako M, Hirai F, et al. Efficacy of Granulocyte and Monocyte Adsorptive Apheresis in Patients With Inflammatory Bowel Disease Showing Lost Response to Infliximab. J Crohns Colitis. 2020;14(9):1264-73.

43. Nakamura M, Yamamura T, Maeda K, Sawada T, Mizutani Y, Ishikawa E, et al. Refractory Ulcerative Colitis Improved by Scheduled Combination Therapy of Vedolizumab and Granulocyte and Monocyte Adsorptive Apheresis. Internal medicine (Tokyo, Japan). 2020;59(23):3009-14.

44. Rodríguez-Lago I, Sempere L, Gutiérrez A, Núñez A, Leo Carnerero E, Hinojosa E, et al. Granulocyte-monocyte apheresis: an alternative combination therapy after loss of response to anti-TNF agents in ulcerative colitis. Scandinavian journal of gastroenterology. 2019;54(4):459-64.

15 Figures and tables

Figure 1: PRISMA flow chart representing the process of the study search and selection

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Table 1: Characteristics of included studies

Supplementary Figure 1: Risk of bias assessment on study level in studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 2: Risk of bias assessment across studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 3: Forest plot of studies comparing clinical remission induction or clinical improvement between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 4: Subgroup analysis of studies comparing clinical remission induction or clinical improvement after 12 weeks between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 5: Subgroup analysis based on criteria of remission in studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 6: Forest plot of studies comparing frequency of adverse events between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 7: Results of Trial Sequential Analysis. A: clinical remission induction, B: clinical remission maintenance, C: Clinical remission induction based on remission criteria, D: Clinical remission induction or clinical improvement

Supplementary Table 1: List of reported adverse events.

Supplementary Table 2: Certainty of evidence by GRADE approach

Table 1: Characteristics of included studies

*** All patients received standard of care added to investigator/comparator. 1: one patient was excluded from analysis because of protocol deviations; 2: one patient was excluded from analysis because of protocol deviations; 3: one patient was excluded due to failure to return blood from the column; 4: minimization may be implemented without a random element, and this is considered to be equivalent to being random. Abbreviations: GMA=granulocyte/monocyte apheresis; n= number; CAI=Clinical Activity Index; EI=Endoscopic Index; 5-ASA=5-aminosalicylic acid; AZA=azathioprine; 6-MP=6-mercaptopurine;**

Clinical remission induction										
Study Name and Setting	**N⁰ of cycles (n)**	**Randomization***	**N⁰ of patients analyzed (n)**	**Patients achieving response**		**Patients achieving remission**		**Time of assessment**	**Outcome criteria**		**Concomitant medication**
				%	**n**	**%**	**n**		**Remission**	**Response**	
Bresci 2008 single center study	5	GMA	40	92.5	37	72.5	29	5 weeks	CAI<6; EI<4	CAI<6; EI>4	oral 5-ASA
		steroid	40	65.0	26	50.0	20				
Doménech 2018 multi-center study	7	GMA+steroid	62 [1]	58.1	36	19.4	12	12 weeks	Mayo ≤2 and no steroid use	Mayo score decrease ≥3 or at least 30% from baseline	stable dose AZA and steroid were allowed if started before randomization
		steroid	61 [2]	49.2	30	18.0	11				
Eberhardson 2017 single center study	5	GMA	14	57.1	8	35.7	5	12 days	Mayo score ≤3	Mayo score decrease ≥3 or at least 30% from baseline	stable dose of steroid; 5-ASA and/or thiopurines were allowed
		sham	8 [3]	37.5	3	12.5	1				
Hanai 2004 single center study	7	GMA	46	93.5	43	82.6	38	12 weeks	CAI≤4	CAI had fallen, but still 4<	steroids and/or 5-ASA
		steroid	23	78.3	18	65.2	15				
Hanai 2008 multi-center study	11	GMA	35	80.0	28	74.3	26	12 weeks	CAI≤4	CAI decreased by ≥5 points, but remained ≥5	all patients were on salicylates and the majority were on low dose steroid as well
		steroid	35	62.9	22	48.6	17				

Nakamura 2004 single center study	5	GMA	10	N/A	N/A	80.0	8	6 weeks	based on CAI, but not specified		all patients received steroid; 5-ASA was unchanged
		no GMA	10	N/A	N/A	20.0	2				
Sands 2008 A study multi-center study	10	GMA	31	67.7	21	16.1	5	12 weeks	Mayo score ≤2; 0-1 endoscopic score	Mayo score decrease ≥3	one or more of the following: 5-ASA agents, steroid, 6-MP or AZA
		sham	16	62.5	10	18.8	3				
Sands 2008 B study multi-center study	10	GMA	112	60.7	68	17.0	19	12 weeks	Mayo score ≤2; 0-1 endoscopic score	Mayo score decrease ≥3	one or more of the following: 5-ASA, steroid, 6-MP or AZA
		sham	56	50.0	28	10.7	6				
Sawada 2005[4] multi-center study	7	GMA	10	80.0	8	20.0	2	10 weeks	CAI=0	CAI improved >3	except for steroid, other medications remained unchanged
		sham	9	33.3	3	11.1	1				

Clinical remission maintenance

Study Name	Number of cycles (n)	Randomization	Number of patients analyzed (n)	Number of patients in clinical remission at the end of the study		Close-out examination	Outcome criteria for remission	Concomitant medication
				%	n			
Emmrich 2006 sigle center study	5	GMA	8	62.5	5	6 months	CAI≤4	all patients were on steroid; 5-ASA was allowed; AZA given at baseline remained unchanged
		no GMA	5	20.0	1			
Fukunaga 2012 single center study	12	GMA	10	40.0	4	12 months	CAI≤4	stable dose of AZA and steroids were allowed if started before randomization
		sham	11	9.1	1			
Maiden 2008 single center study	5	GMA	18	77.8	14	6 months	CAI≤6	only 5-ASA or oral steroid
		no GMA	19	26.3	5			

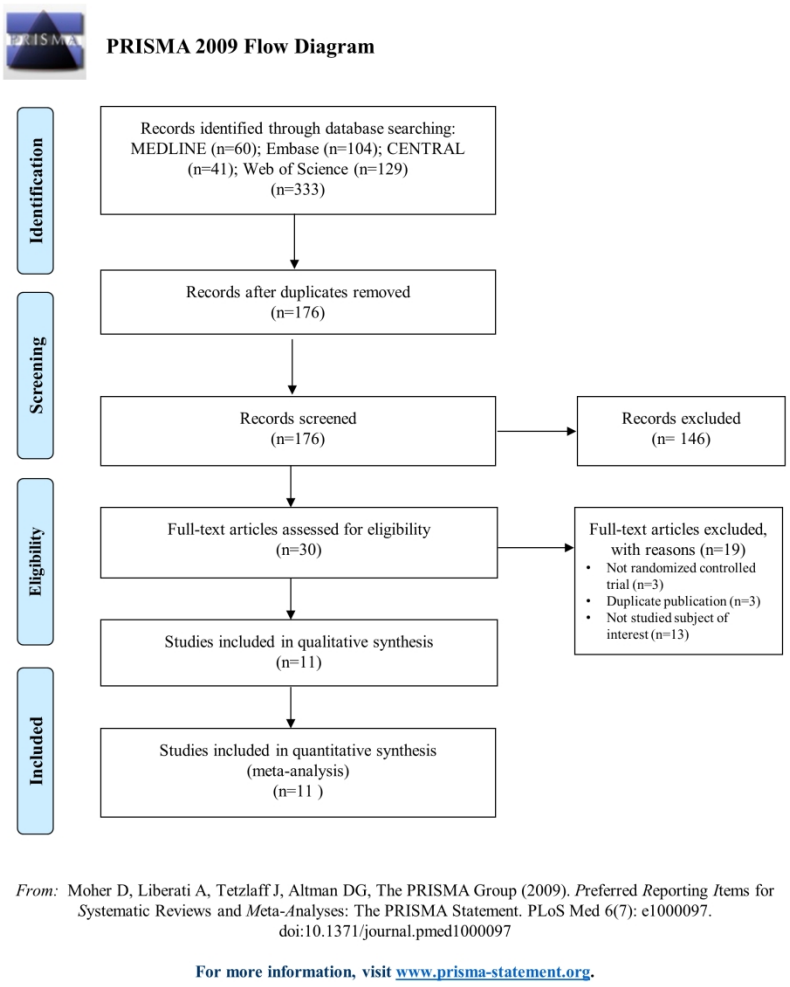

Figure 1: PRISMA flow chart representing the process of the study search and selection

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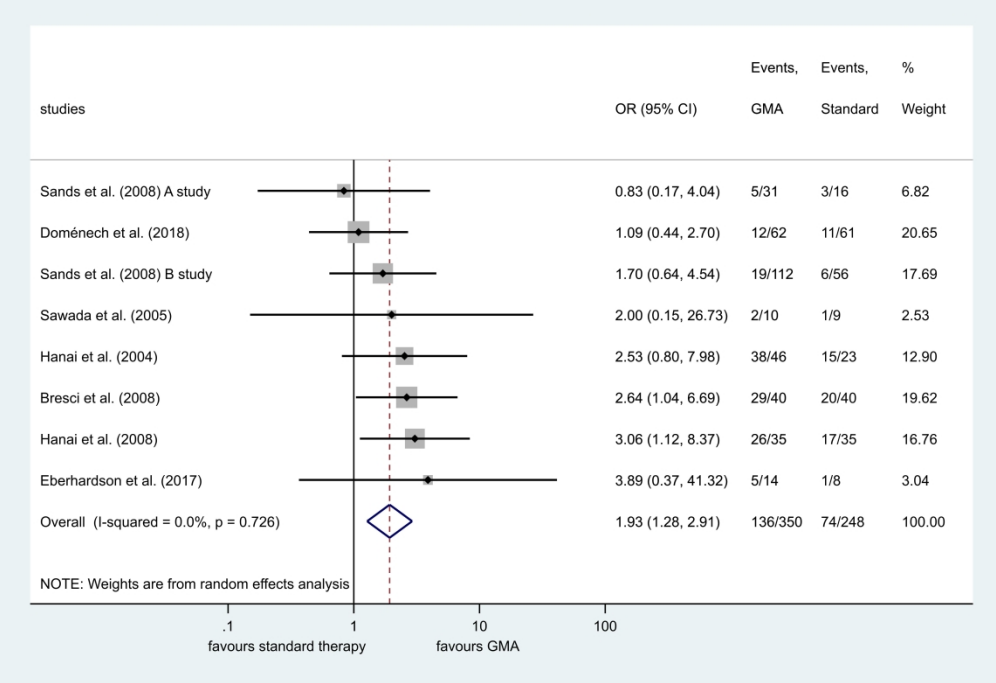

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

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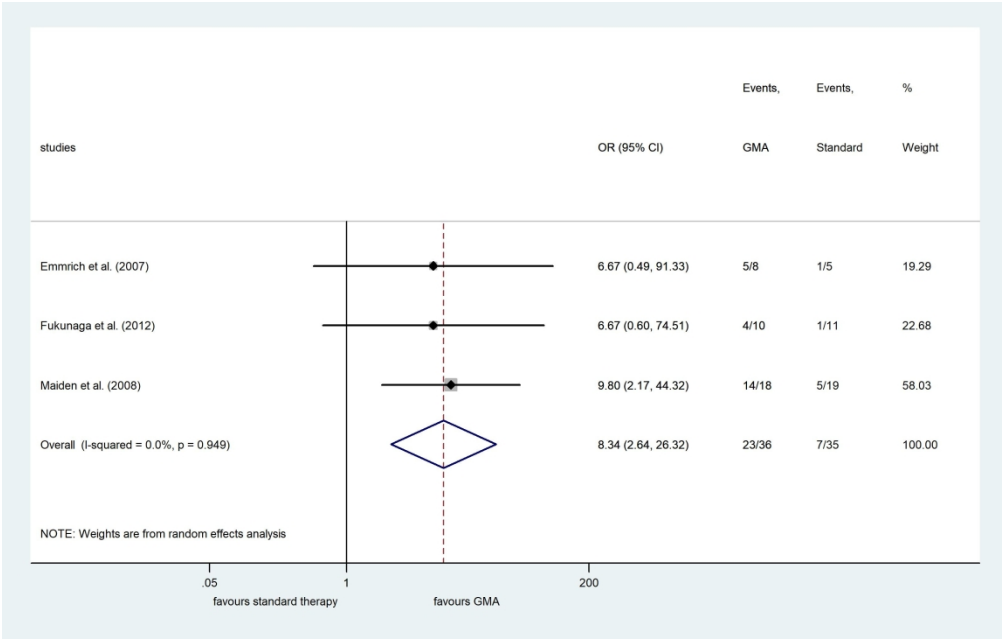

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

137x87mm (600 x 600 DPI)

Supplemetary material

Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

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Keywords: Inflammatory bowel disease

Search strategy for MEDLINE database

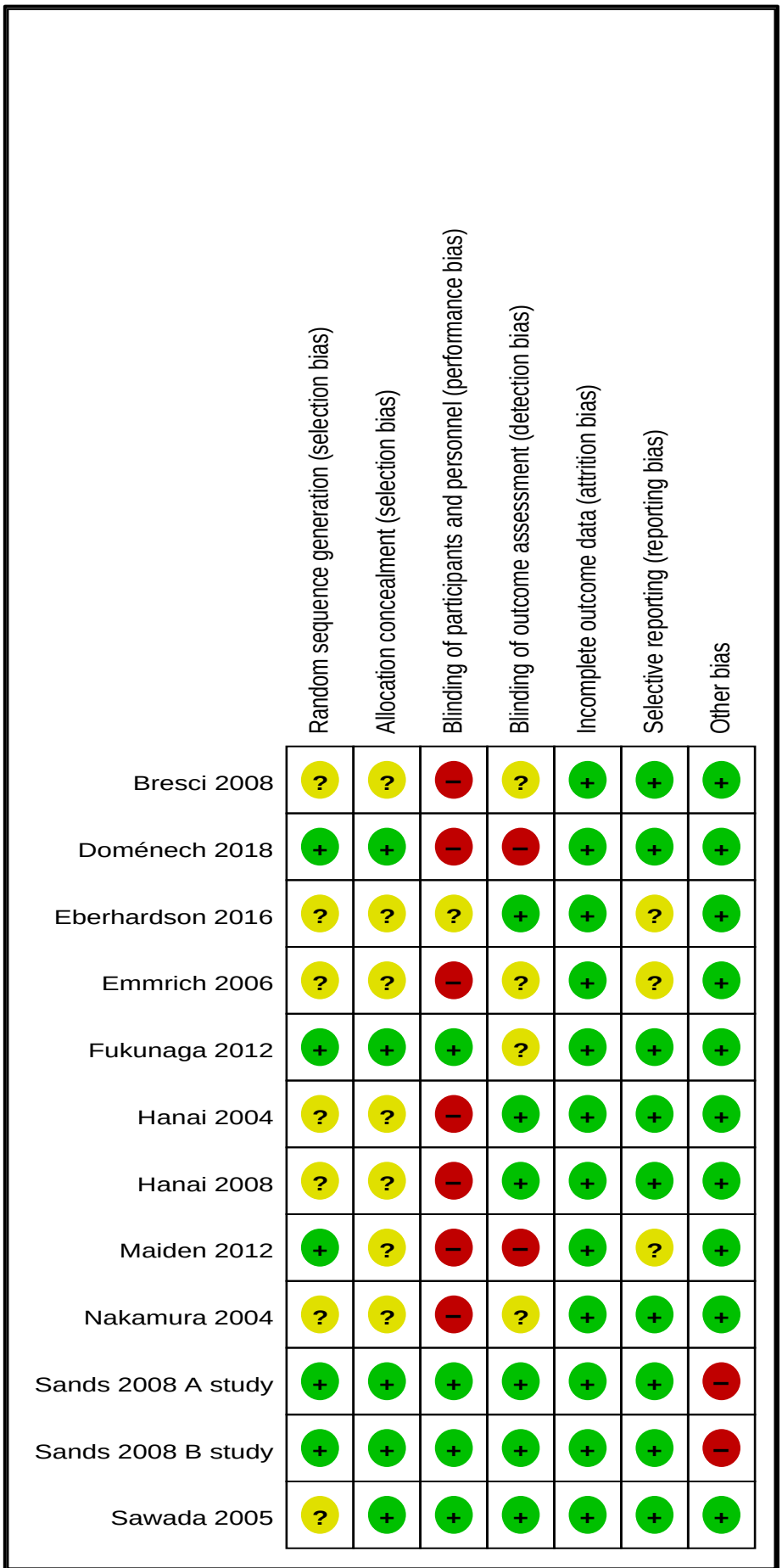
Date of search: 5th March, 2019

Full query: (gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND ("inflammatory bowel disease" OR "ulcerative colitis") AND (random*)

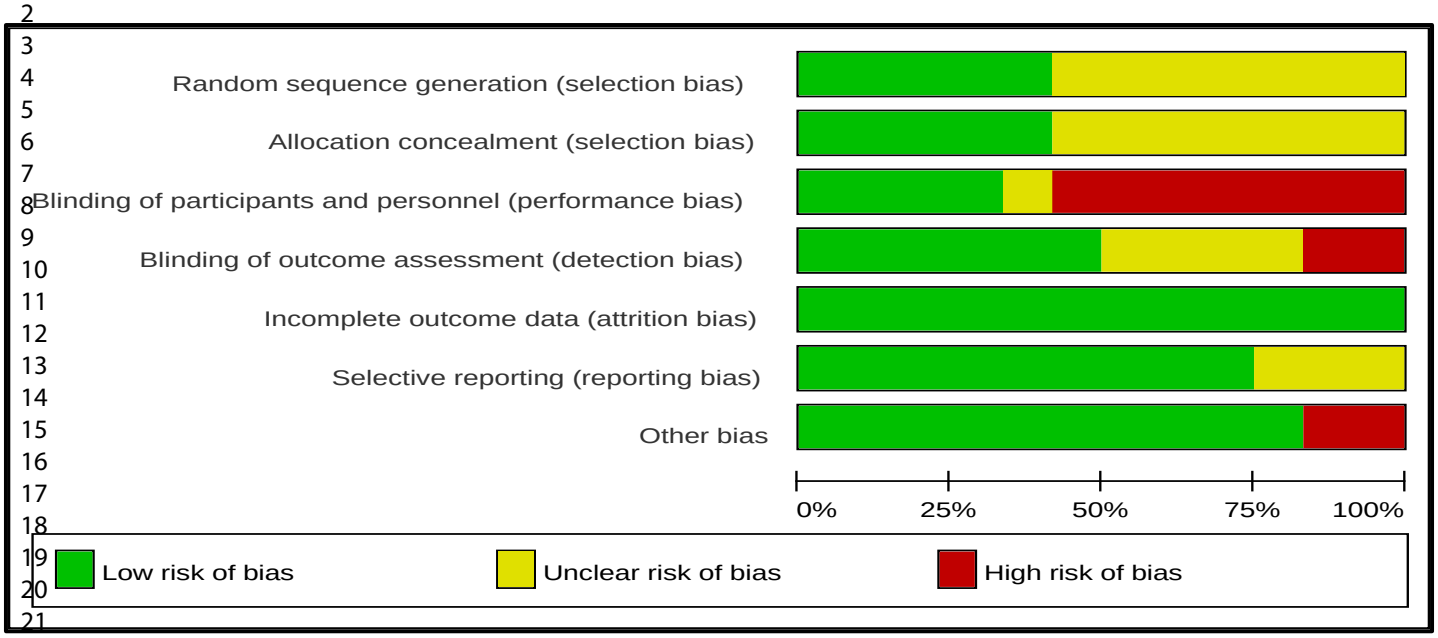
No filters or restrictions were applied.

Search	Query	Automatic explosion
#1	gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]
#2	"inflammatory bowel disease" OR "ulcerative colitis"	"inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields]
#3	random*	"random*"[All Fields]
#4	#1 AND #2	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]) AND ("inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields])
#5	#3 AND #4	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]) AND ("inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields]) AND "random*"[All Fields]

Supplementary Figure 1



Supplementary Figure 2



Detailed risk of bias assessment

Bresci et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomized study, but method was not specified in the manuscript
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant data have been reported. Adverse events were adequately reported.
Other bias	Low risk	The study appears to be free of other sources of bias.

Doménech et al. 2018	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...randomizaton codes were centerally generated using a computer procedure..." Blocked randomization was used.
Allocation concealment (selection bias)	Low risk	Quote: "...randomizaton codes were centerally generated using a computer procedure..."
Blinding of participants and personnel (performance bias)	High risk.	Open-label.
Blinding of outcome assessment (detection bias)	High risk	Quote: "...the endoscopist was not necessarily blinded..."
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat method was used. 123/125 patients completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported. Adequate

		description of adverse events.
Other bias	Low risk	The study appears to be free of other sources of bias.

Eberhardson et al. 2017	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Blocked randomization (3:2), but method is fully specified.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	Unclear risk	Double-blind, but insufficient data to permit judgement (form of placebo treatment was not described).
Blinding of outcome assessment (detection bias)	Low risk	Quote: "The FACS analysis was blinded to the clinical participants and the FACS analyst was also blinded before unblinding day 12."
Incomplete outcome data (attrition bias)	Low risk	1/9 patient from the placebo group was excluded from the study just after the randomization because of SADE (failure to return blood from the column). 2/14 (14%) were excluded from active study group because of adverse event and worsening of the disease, but analysis was conducted on full analyses set basis.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate.
Other bias	Low risk	The study appears to be free of other sources of bias.

Hanai et al. 2004	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized study, but method was not specified in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but other similar article from the authors was stated as unblinded.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Each patient was assessed blindly..."

Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Low risk	The study appears to be free of other sources of bias.

Hanai et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized study, but method is not described in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Stated as unblinded.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Each patient was assessed blindly..."
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	Low risk	The study appears to be free of other sources of bias.

Nakamura et al. 2004	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized, but the method was not specified in the manuscript
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	No information
Incomplete outcome data (attrition bias)	Low risk	60/66 completed the study; 1 took non-permitted drugs,1 relapsed just after the randomization, further 4 withdrew the consent.

Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	Low risk	The study appears to be free of other sources of bias.

Sands et al. 2008 A study	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...using sealed envelopes with sequential numbers issued in blocks of 3..." and
Allocation concealment (selection bias)	Low risk	Quote: "...using sealed envelopes with sequential numbers issued in blocks of 3..." and
Blinding of participants and personnel (performance bias)	Low risk	Quote: "a polyvinylchloride bypass tube was inserted between the Adacolumn and the Adacircuit to permit bypass of the column among patients undergoing sham procedures."
Blinding of outcome assessment (detection bias)	Low risk	The gastroenterology team was blinded to the treatment assignment.
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat analysis; however, 66% of patients completed the study (6 patients left the study because of disease flare; 5 from apheresis group, 1 from sham group).
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	High risk	Quote: "Subjects who withdrew before the week 12 visit were treated as treatment failure for primary end point (clinical remission)." Comment: these imputation of ITT analysis may cause bias.

Sands et al. 2008 B study	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Randomization was performed according to a computer-generated scheme

		that used an integrated voice response system."
Allocation concealment (selection bias)	Low risk	Quote: "Randomization was performed according to a computer-generated scheme that used an integrated voice response system."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "a polyvinylchloride bypass tube was inserted between the Adacolumn and the Adacircuit to permit bypass of the column among patients undergoing sham procedures."
Blinding of outcome assessment (detection bias)	Low risk	The gastroenterology team was blinded to the treatment assignment.
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat analysis; however, 66% of patients completed the study (6 patients left the study because of disease flare; 5 from apheresis group, 1 from sham group).
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	High risk	Quote: "Subjects who withdrew before the week 12 visit were treated as treatment failure for primary end point (clinical remission)." Comment: these imputation of ITT analysis may cause bias.

Sawada et al. 2005	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	minimization by an independent controller.
Allocation concealment (selection bias)	Unclear risk	Quote: “The assignment of the enrolled patients to the active group or the sham group was performed by a controller who was independent of the other staff, patients, and relatives.”
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Both columns were covered with an opaque material so that they could

		not be distinguished by the patients."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "To ensure proper blinding within the clinical evaluation, the medical staffs of each institution were separated into two independent groups."
Incomplete outcome data (attrition bias)	Low risk	All of the enrolled eligible patients were evaluated.
Selective reporting (reporting bias)	Low risk	All outcomes of interest were reported.
Other bias	Low risk	The study appears to be free of other sources of bias. Comment: these imputation of ITT analysis may cause bias.

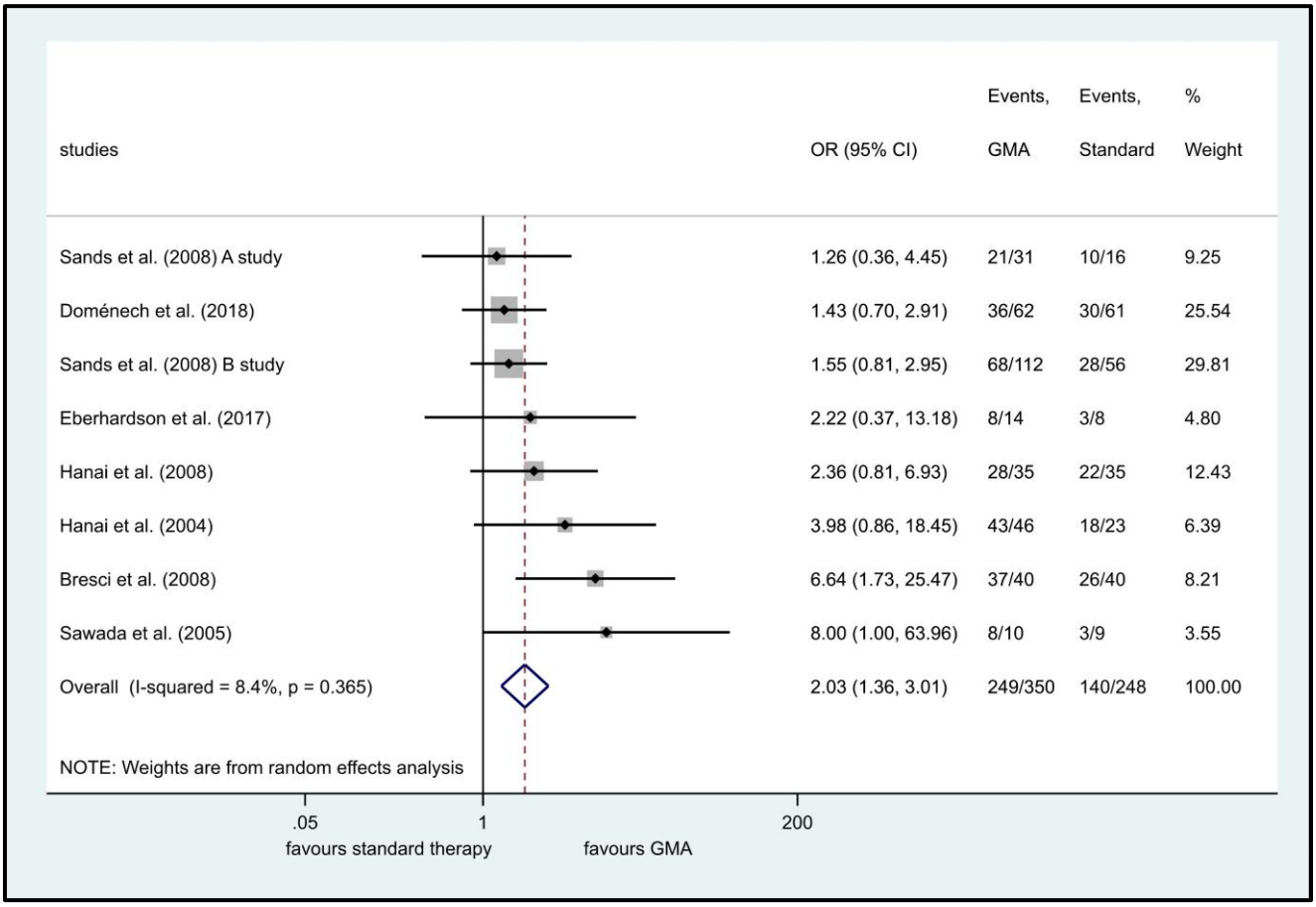
Emmrich et al. 2006	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized, but method is not specified in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	Only 1/9 patient from active group discontinued the study.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate.
Other bias	Low risk	The study appears to be free of other sources of bias.

Fukunaga et al. 2012	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Blocked randomization according to a computer-generated scheme.
Allocation concealment (selection bias)	Low risk	Patients were randomized in a 1:1:1 ratio by a statistician at an independent organization.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Both patients and the physician were blinded by a curtain."

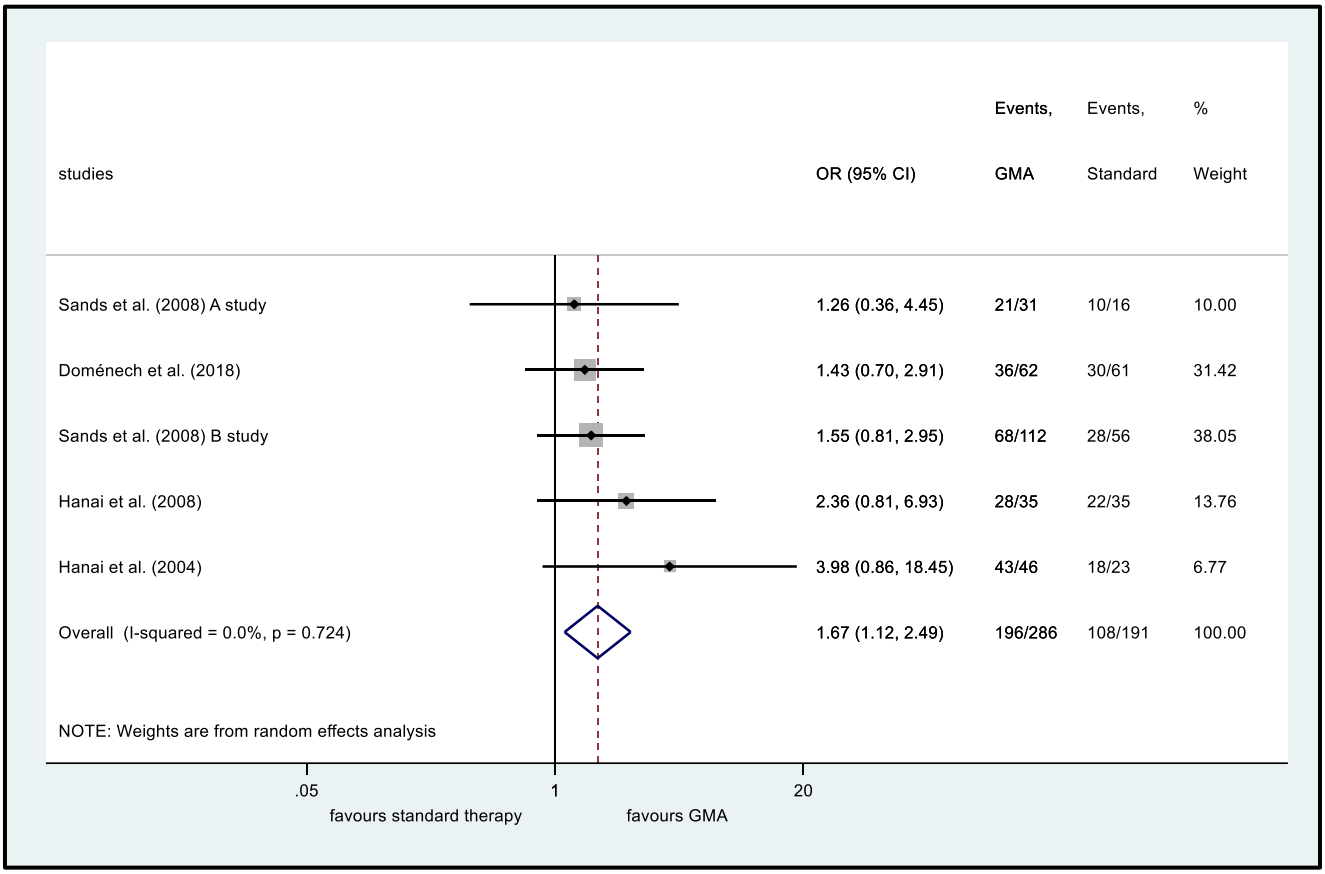
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	21/22 completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Unclear risk	Concomitant therapeutic regimen was not described clearly, and the authors stated: "a significant fraction of patients in each arm were on concomitant PSL or AZA and this enabled us to assess the contribution of these medications"

Maiden et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Randomization was conducted using a linear random number generator of 0 to 1."
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Open label
Blinding of outcome assessment (detection bias)	High risk	Open label
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate. Number of events in the control group was not described.
Other bias	Low risk	The study appears to be free of other sources of bias.

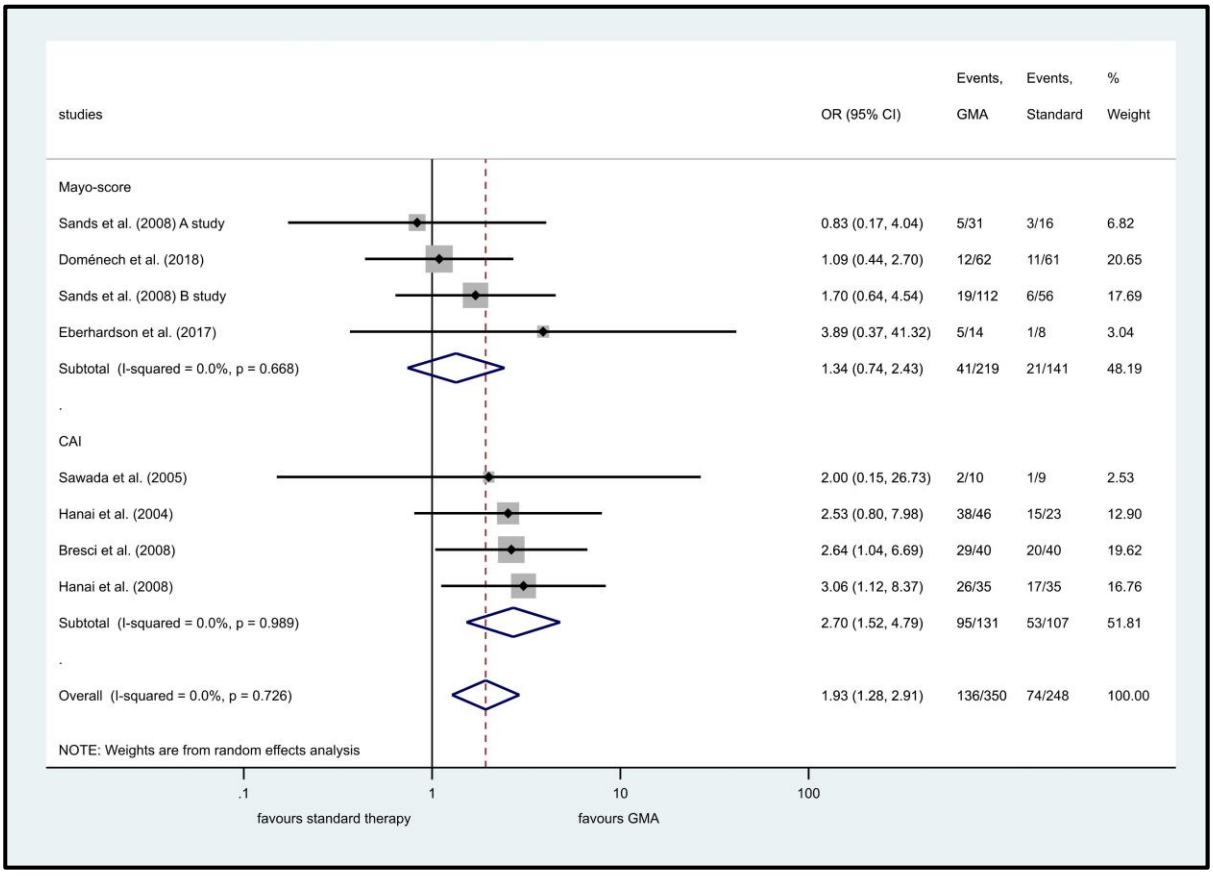
Supplementary Figure 3



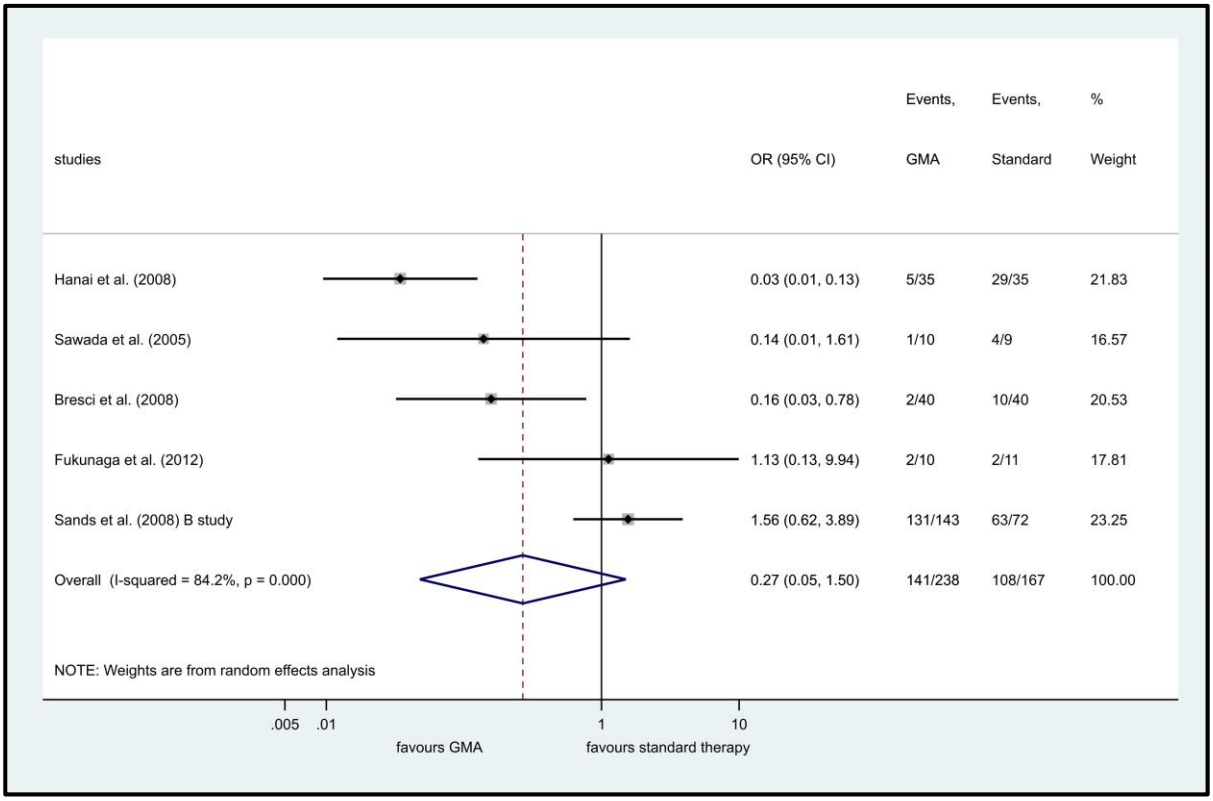
Supplementary Figure 4



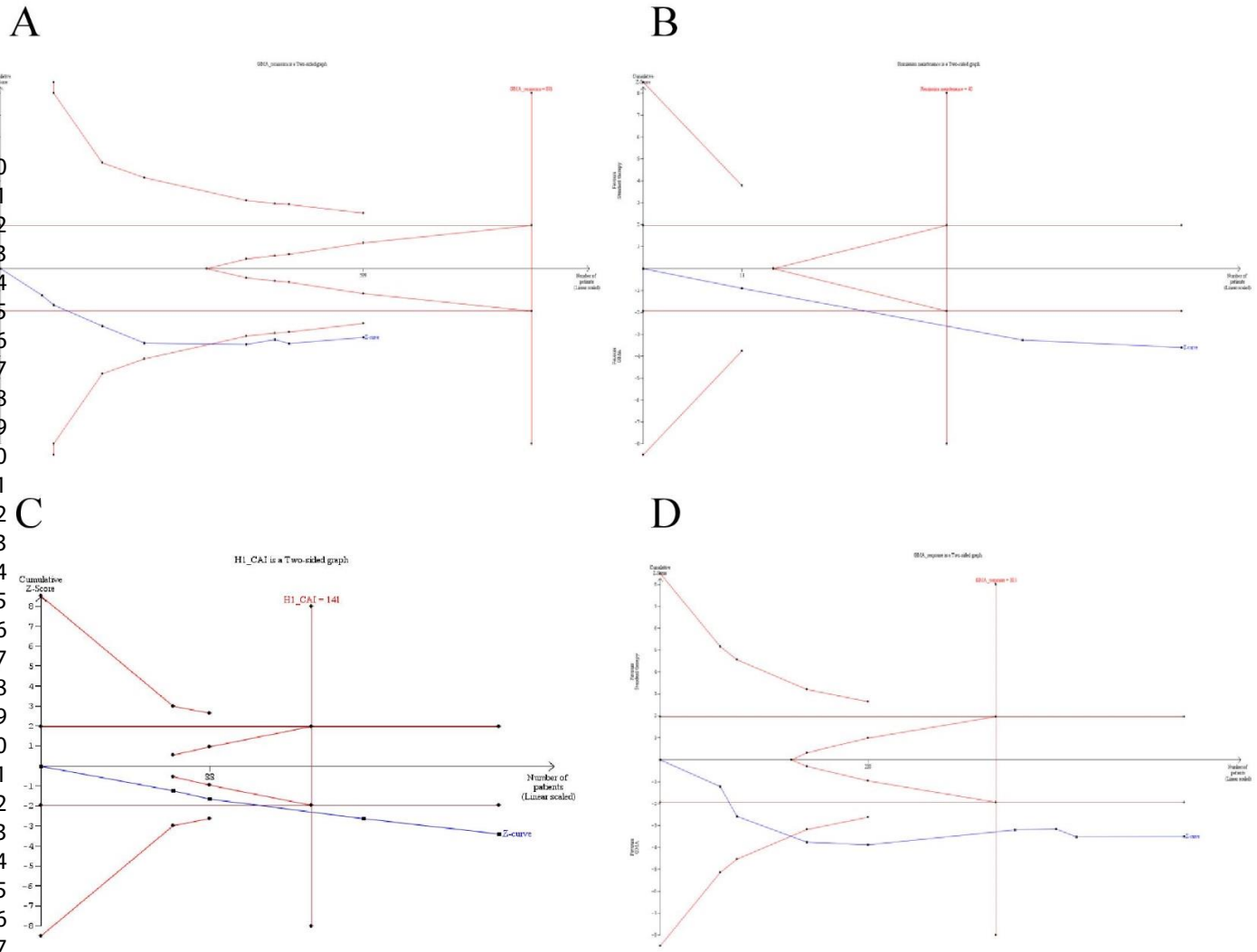
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Table 1

Study	Reported adverse events
Hanai et al. 2004	flushing, nausea, mild fever
Sawada et al. 2005	fever, skin rash, back pain
Bresci et al. 2008	headache, gastrointestinal intolerance, facies lunaris, vascular hypertension, glucose intolerance
Fukunaga et al. 2012	nausea, skin itchiness
Sands et al. 2008	headache, disease flare-up, decreased diastolic blood pressure, nasopharyngitis, hypotension, nausea, fatigue, post procedure hematoma, abdominal pain, dizziness, vomiting, vessel puncture site bruise, diarrhea, upper respiratory tract infection, flatulence

Supplementary Table 2

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	standard therapy for clinical remission induction and GMA as an adjunctive therapy	standard therapy for clinical remission induction	Relative (95% CI)	Absolute (95% CI)		
Clinical remission rate (assessed with: CAI or Mayo-score)												
8	randomized trials	very serious	not serious	not serious	serious	none	136/350 (38.9%)	74/249 (29.7%)	**OR 1.94** (1.28 to 2.92)	**153 more per 1 000** (from 54 more to 255 more)	⊕◯◯◯ VERY LOW	CRITICAL
Clinical response and clinical improvement (CAI or Mayo-score)												
8	randomized trials	very serious	not serious	not serious	not serious	none	249/350 (71.1%)	140/249 (56.2%)	**OR 2.05** (1.37 to 3.06)	**162 more per 1 000** (from 75 more to 235 more)	⊕⊕◯◯ LOW	CRITICAL
Clinical remission maintenance rate (assessed with: CAI)												
3	randomized trials	serious	not serious	serious [a]	not serious	none	39/36 (108.3%)	17/35 (48.6%)	**OR 8.34** (2.64 to 26.32)	**402 more per 1 000** (from 228 more to 476 more)	⊕⊕◯◯ LOW	CRITICAL
Adverse events												
5	randomized trials	very serious	not serious	very serious [b]	very serious [c,d]	publication bias strongly suspected	141/238 (59.2%)	108/167 (64.7%)	**OR 0.27** (0.05 to 1.50)	**316 fewer per 1 000** (from 563 fewer to 86 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Steroid-sparing effect												
3	randomized trials	serious	not serious	not serious	very serious [d]	none	66	43	-	WMD **6.83 mg/day lower** (14.47 lower to 0.81 higher)	⊕◯◯◯ VERY LOW	IMPORTANT

CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Duration of follow-up differs among studies (6 months or 12 months). b. Pool of adverse events differs among studies. c. The optimal information size criterion is not met. d. TSA could not be carried out.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2, 3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3, 4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Suppl.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4, 5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5, 6, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6, Suppl. Figure 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6, Figure 2-3, Suppl. Figure 4-5
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency	9, Figure 2-3, Suppl. Figure 3, Suppl. Figure 6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Suppl. Figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Suppl. Figure 4-5; Suppl Figure 7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Suppl. Table 1

PRISMA 2009 Checklist

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	2, 9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Haematology (incl blood transfusion)
Keywords:	Inflammatory bowel disease < GASTROENTEROLOGY, IMMUNOLOGY, Gastroenterology < INTERNAL MEDICINE, HAEMATOLOGY

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Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

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Keywords: Inflammatory bowel disease; IMMUNOLOGY; Gastroenterology; HAEMATOLOGY

1 Abstract

Objective: The goal of treatment in ulcerative colitis (UC) is to induce and maintain remission. The addition of granulocyte and monocyte apheresis (GMA) to conventional therapy may be a promising therapeutic alternative. In this meta-analysis, we aimed to assess the efficacy and safety profile of GMA as an adjunctive therapy.

Design: Systematic review and meta-analysis.

Methods: We searched four databases (MEDLINE, Embase, Web of Science, and Cochrane Central Register of Controlled Trials) for randomized or minimized controlled trials which discussed the impact of additional GMA therapy on clinical remission induction and clinical remission maintenance compared to conventional therapy alone. Primary outcome were clinical remission induction and maintenance, secondary outcomes were adverse events and steroid-sparing effect. Odds ratios (OR) with 95% confidence intervals were calculated. Trial Sequential Analyses (TSA) were performed to adjusts for the risk of random errors in meta-analyses.

Results: A total of eleven studies were eligible for meta-analysis. GMA was clearly demonstrated to induce and maintain clinical remission more effectively than conventional therapy alone (598 patients: OR: 1.93, CI: 1.28–2.91, p=0.002, I^2=0.0% for induction; 71 patients: OR: 8.34, CI: 2.64–26.32, p<0.001, I^2=0.0% for maintenance). There was no statistically significant difference in the number of adverse events (OR: 0.27, CI: 0.05–1.50, p=0.135, I^2=84.2%)

Conclusion: GMA appears to be more effective as an adjunctive treatment in inducing and maintaining remission in UC patients than conventional therapy alone.

Protocol registration number: PROSPERO CRD42019134050.

Word count: 4199

2 Article Summary

Strengths and limitations of this study

- This is the first meta-analysis assessing the role of GMA in clinical remission maintenance in ulcerative colitis.
- Grading of Recommendations Assessment, Development and Evaluation approach was applied to appraise the certainty of evidence.
- Our results are limited by the relatively low number of patients and the heterogenous reporting of adverse events.
- To address the limitation by the number of included patients and to control both type I and type II errors, Trial Sequential Analyses have been performed.

3 Introduction

Ulcerative colitis (UC) is one of two major types of inflammatory bowel disease (IBD). The incidence of this disease varies from nine to 20 cases per 100 000 person-years (1). UC is a lifelong illness that has a profound impact on patients. The primary goal of treatment is to achieve and maintain remission, thereby preventing colectomy and colorectal neoplasms and ensuring an acceptable quality of life (2). The choice of treatment for patients with UC is tied to the clinical and endoscopic severity of the disease along with the frequency and severity of relapses. Patients with no response to conventional therapies, especially to corticosteroids and immunosuppressive agents, are common candidates for biological treatments and/or surgery. However, both of these options are challenged by the high costs and incidence of side-effects and complications.

Patients with UC usually have a raised level of granulocytes, and, in the case of an active disease, the mucosa of the bowel is infiltrated by a large number of granulocytes and macrophages. These leukocytes release degradative enzymes and proinflammatory cytokines, which lead to further inflammation of the bowel. Based on the hypothesis that a reduction of activated granulocytes and monocytes/macrophages may be beneficial, granulocyte-monocyte apheresis (GMA) was proposed as a strategy to promote remission in active UC (3). GMA is a novel non-pharmacological treatment tool for patients with UC, comprising an extra-corporeal absorptive circuit, which decreases inflammatory cytokines and upregulates regulatory T cells. Despite its high cost, GMA seems to have a good safety profile (3).

However, data on the efficacy of GMA are still debated. The first studies published in Japan showed remission or response rates of up to 60–80% (4-6). Sands et al. reported a study with a large number of patients comparing GMA to a placebo, and they found no significant difference in terms of clinical response (7). This substantial difference between studies could be explained by the heterogeneity of patients' characteristics, most probably by the varying severity and extent of the disease.

A large proportion of patients require long-term, high-dose steroid treatment, which often results in severe side-effects impairing patients' quality of life. If addition of GMA can reduce the dose of corticosteroids, the risk of steroid-induced adverse events (AEs) could be minimized. Therefore, it is also essential to evaluate the steroid-sparing effects of GMA (8). Beyond the induction of remission and the impact on steroid requirement, the role of GMA in maintaining remission is unclear (9). The aim of our study was to assess the role of GMA in the induction and maintenance of clinical remission in UC and to evaluate the potential steroid-sparing effect of the therapy.

4 Methods

The meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (10). The review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews (CRD42019134050).

4.1 Search strategy

The systematic literature search was conducted by two independent reviewers (KS and FM) in MEDLINE (via PubMed), EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science for studies published up to 5th March 2019. The search query in each database was based on PICO components combined with Boolean operators: (gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND ("inflammatory

bowel disease" OR "ulcerative colitis") AND (random*). Details of our search strategy and terms are presented in supplementary material.

4.2 Eligibility criteria

General criteria: a randomized controlled trial (RCT) or minimized controlled trial (This type of sequence generation is considered to be nearly equivalent to being random) (11); only full-text articles were included.

Specific criteria for clinical remission induction: patients with active UC ($Population_1$), standard therapy for remission induction and GMA as an adjunctive therapy ($Intervention_1$), and standard therapy for remission induction ($Comparison_1$); $Outcomes_1$: clinical response rate and clinical remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.

Specific criteria for clinical remission maintenance: patients with UC in clinical remission induced by GMA ($Population_2$), standard therapy for remission maintenance and GMA as an adjunctive therapy ($Intervention_2$), and standard therapy for remission maintenance ($Comparison_2$); $Outcomes_2$: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

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.

The titles of the studies were screened based on predefined criteria, and the relevant studies were selected for abstract review. If the abstract was found to be appropriate, the full text of the article was studied. The decision to include a study in the meta-analysis was based on an independent assessment by the two reviewers and eventually by consensus for resolution of any disagreements. Reference lists in included studies and reviews on this topic were searched for additional studies. Publications citing the included studies were also screened in the Google Scholar academic search engine.

4.3 Data extraction

The two investigators (KS and FM) reviewed the articles independently and extracted data into a standardized data collection form (discrepancies were resolved based on consensus). For the selected studies, characteristics were extracted, including publication year, country, number of centres, number of patients, and study design. In addition, patient characteristics (age, sex, and extent of disease), details of therapy (concomitant medication, volume of GMA, number of GMA cycles, and duration of treatment), and main outcomes (number of patients with clinical improvement/response, number of patients achieving clinical remission, number of patients with maintained remission, and number of AEs) were also extracted.

4.4 Risk of bias assessment

The Cochrane Risk of Bias Tool was used by the two independent investigators (KS and FM) to assess the quality of the studies included. Any disagreement was resolved based on consensus (12). Major domains of quality assessment were the following:

1. Random sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)

5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)
7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials, and imputation of intention-to-treat (ITT) analysis)

4.5 Statistical analysis

The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR) with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered statistically significant (except for heterogeneity, for which a p value <0.10 was considered significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR, WMD, and weights are presented in forest plots. The random-effects model was used to pool effect sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating Higgins' I^2 indicator (13, 14). The Q statistics were computed as the squared deviations from the pooled effect of the weighted sum of individual study effects, with the weights being used in the pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees of freedom (where k was the number of studies). The I^2 index corresponds to the percentage of the total variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–60%), substantial (50–90%), and considerable (75–100%) (11). Subgroup analysis was performed as described in the study protocol if a sufficient number of studies was available. Funnel plots were used to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal information size (OIS). This methodology combines an information size with the threshold of statistical significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).

4.6 Quality of evidence

The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality of evidence for each outcome (15, 16). Disagreements were resolved by consensus.

5 Results

5.1 Search and selection

The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After screening and assessment for eligibility, eleven full-text articles containing one minimized controlled trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active UC, and three studies contained data on patients with UC in clinical remission.

5.2 Characteristics of the studies included

The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients received GMA, and 248 were in control groups. All the participants had active UC and were treated with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received standard of care added to the intervention/comparator and they did not receive any anti-TNF agent.

Both GMA and control were added to conventional treatment. In terms of main outcomes, the studies investigated the rate of clinical remission and clinical response. Investigators assessed the activity of UC with either the Mayo score or CAI. One study required steroid-free remission to regard cases as being in clinical remission.

In the case of clinical remission maintenance, all the studies were randomized controlled trials. A total of 71 participants (mean: 24, ranging from 13 to 37) were included in this meta-analysis: 36 patients received GMA, and 35 were in control groups. All the participants had ulcerative colitis in remission and were treated with Adacolumn® or Cellsorba®. One trial evaluated GMA vs sham control (24) and two trials assessed GMA compared to standard therapy alone (9, 25). Both GMA and sham control were added to conventional treatment. In terms of main outcome, the studies investigated the rate of clinical relapse.

Three studies also reported on the steroid-sparing effect of GMA (9, 17, 22).

5.3 Risk of bias assessment

A summary of risk of bias assessment is shown in **Supplementary Figure 1 and Supplementary Figure 2**. Three unblinded studies were at high risk of performance bias (19, 22, 25). Because of the nature of the intervention, four studies which lacked a description of the blinding process were interpreted as having a high risk of bias (18, 21, 23, 24). As regards assessment blinding, two unblinded studies were judged to be at high risk of bias (19, 25). Two studies were deemed as having a high risk of other bias; although they used ITT analysis, they considered subjects who left the study as a treatment failure that may lead to bias (7).

5.4 Efficacy and safety of GMA in clinical remission induction

Seven randomized and one minimized controlled trial evaluated clinical remission induction. GMA therapy was associated with a better clinical response rate compared to the control group (OR = 2.03, 95% CI = 1.36–3.01, p<0.001, I^2 = 8.4%) (**Supplementary Figure 3**). Subgroup analysis of studies with assessment at 12 weeks also showed benefit (OR = 1.67, 95% CI = 1.12–2.49, p=0.012, I^2 = 0.0%) **(Supplementary Figure 4)**. Patients undergoing GMA therapy had a higher remission rate compared to standard therapy without GMA (OR = 1.93, 95% CI = 1.28–2.91, p=0.002, I^2 = 0.0%) (**Figure 2**). Sub-group analyses were performed based on activity indices and number of GMA cycles. No difference was found between the two groups in studies assessing UC with the Mayo score (OR = 1.34, 95% CI = 0.74–2.43, p=0.334, I^2 = 0.0%), but the remission induction was more successful in studies using CAI for assessment (OR = 2.70, 95% CI = 1.52–4.79, p=0.001, I^2 = 0.0%) (**Supplementary Figure 5**). A significant difference was found in studies using five cycles compared to the control (OR = 2.78, 95% CI = 1.17–6.60, p=0.021, I^2 = 0.0%) and more than five cycles compared to standard therapy alone (OR = 1.73, 95% CI = 1.08–2.77, p=0.022, I^2 = 0.0%). There was no statistically significant difference in the number of AEs (p=0.135) (**Supplementary Figure 6**). No statistically significant steroid-sparing effect was detected among patients with active UC (p=0.080). A list of reported AEs is presented in **Supplementary Table 1**.

5.5 Efficacy and safety of GMA in clinical remission maintenance

Three randomized clinical trials evaluated the clinical remission rate in remitting UC induced by GMA. Patients receiving GMA had a higher rate of clinical remission maintenance (OR = 8.34, 95% CI = 2.64–26.32, p<0.001, I^2 = 0.0%) (**Figure 3**). Due to lack of data, the rate of AEs could not be assessed in this population.

5.6 Trial Sequential Analysis

Based on a TSA, the cumulative Z curve crossed the trial sequential significance boundary as regards clinical remission induction and clinical remission maintenance (power=80.0%; alpha=5.0%) (**Supplementary Figure 7**). Moreover, clinical remission maintenance exceeded the required meta-analysis sample size, possibly suggesting that further clinical trials are not required. A TSA for AEs and steroid-sparing effects could not be carried out due to insufficient information size.

5.7 Quality of evidence

The GRADE analysis rated the quality of evidence for primary and secondary outcomes at a very low to low level. GRADE evidence profile is shown in **Supplementary Table 2**.

6 Discussion

The main goal of care is to achieve and maintain remission of UC. This condition is usually treated by a step-up approach, during which treatments are switched or additional treatment is administered to optimize current therapy. There are several therapeutic agents to slow down the clinical activity of UC. Corticosteroids, 5-aminosalicylates, immunosuppressive agents, and tumour necrosis alpha-inhibitors are commonly used, and new therapeutic targets, such as anti-adhesion molecules and anti-interleukins, are emerging. Despite these multiple therapeutic options, there is still a need to expand the scope of treatment methods due to possible development of intolerance or resistance to current treatments. After running out of treatment options, surgical therapy is frequently the last remaining option for patients. GMA is a novel non-pharmacologic treatment option for active and remitting UC, by which activated granulocytes and monocytes are removed from the circulation. These cells may contribute to the pathogenesis of UC.

Guidelines describing the role of GMA in UC are in agreement on the potential beneficial effect and favourable safety profile. They also agree that there is insufficient evidence in this field of practice (26, 27).

To our knowledge, the first report on the efficacy of GMA in UC was published in Japan in 2001 (28). This study found a considerably high remission rate with only five sessions of GMA in patients refractory to conventional drug therapy. Subsequent studies from the early 21st century had similar results (29-31). In 2008, Sands et al. failed to prove a significant difference in clinical remission rate between GMA and a placebo on a relatively large population (7). However, this study was not free of attrition bias; a high proportion of patients were lost to follow-up. Three systematic reviews and meta-analyses have been conducted in this field so far (32-34). All of them have agreed on the benefit of GMA in clinical remission induction, and they pointed out the necessity for more trials with a rigorous and clear design to further narrow the focus on specific patient groups. These studies used one to three databases for a systematic search and selection.

In our current meta-analysis, a broader literature search was carried out, and the role of GMA in clinical remission maintenance was assessed. Our work supported the hypothesis that GMA improves the rates of clinical response and clinical remission in patients with UC. It should be noted that response and remission rates defined by symptom scores should be cautiously interpreted because they also include subjective elements, such as overall physician judgement on disease activity. A few recent retrospective and prospective studies have suggested certain prognostic factors in the therapeutic response (35-37). It seems that younger patients respond better to GMA therapy, whereas gender and smoking status showed no difference in response to treatment (35). Yokoyama et al. found that shorter duration of UC and lower cumulative corticosteroid dose are associated with a higher efficacy rate

(36). In their study, patients who received GMA treatment immediately after relapse were the best responders. It would be advisable to conduct further research to identify subgroups of UC where patients benefit the most from GMA (38).

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%. To date, there is no uniformly accepted GMA regimen. There are RCTs to compare a ten-cycle and a five-cycle GMA regimen. Dignass et al. and Ricart et al. found similar remission rates between ten and five cycles (46% vs. 36%, p=0.479; 35.7% vs 45.5%, p>0.05, respectively) (38, 39). The latter study also showed a steroid-sparing effect in the group receiving ten cycles of GMA. Sakuraba et al. found that an improved remission rate is associated with intensive GMA (54.0% vs 71.2%, p=0.029 in five- and ten-cycle regimens, respectively) (40). In our meta-analysis, the number of GMA cycles varied among studies as well. We assessed the efficacy of GMA based on the two main regimens in previous trials. Both groups showed a benefit of adding GMA to the therapy compared to standard treatment alone.

Regarding the induction and maintenance of remission, our results relate to clinical remission. In 2015, based on insights from various clinical trials, a new consensus was made on appropriate evidence-based treatment targets (41). From then on, in addition to controlling symptoms, more objective markers came to the fore and endoscopic remission came to the spotlight. Only three of the articles analysed reported a comparison of endoscopic remission. Nakamura et al. found that the improvement in endoscopic score was significantly higher in the group receiving GMA as well (23). Another study showed that the Rachmilewitz's endoscopic index was significantly improved in patients treated with GMA compared to the control group (17). The third study reported similar endoscopic remission rate in the two groups (12% vs 11% in GMA and sham group, respectively; p=1.00) (7). Data on objective inflammatory markers are also contradictory and insufficient (18, 20, 25). In light of this, there is a need for additional, high-quality RCTs that focus on current therapeutic targets.

We found no significant difference between the two groups as regards AEs (very low certainty). Further studies are called for to provide a higher level of evidence on this topic. They would be particularly important for specific subgroups where the safety profile is of paramount importance, such as in cytomegalovirus infection, adolescence, and pregnancy. Clinical trials should also target these populations because fewer therapeutic options are available for them and the safety profile of GMA seems favourable compared to other treatments.

As with any therapeutic option, cost-effectiveness should also be considered. The cost of GMA is much higher compared to regular medication, such as corticosteroids, but GMA could be cost-effective in the long term. The use of GMA may reduce the cost of medical services, hospitalization, and surgery in the long term. Nevertheless, GMA's safety profile is in sharp contrast to multiple severe AEs associated with conventional pharmacologics and biologics. According to recommendations, if UC flares up, treatment is usually escalated to biologics. As GMA and biologics are also likely to differ in terms of invasiveness, safety, and efficacy, the question arises: which one may be more beneficial? However, there is currently no evidence of this. In this regard, limited data are available from recent studies suggesting that GMA may be beneficial in patients who no longer respond to biologics (42-44).

To our knowledge, this is the first meta-analysis to assess the role of GMA in UC remission maintenance. Our study showed that the addition of GMA enhances the proportion of patients who can maintain their remission (low certainty). Fukunaga et al. and Emmrich et al. enrolled clinically active

UC patients based on CAI (9, 24). After successful induction therapy with the inclusion of GMA, patients achieving clinical remission were allocated to groups with and without GMA treatment for remission maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin, which is considered as a risk factor of relapse (25). Their results showed that faecal calprotectin level significantly decreases following five treatment session. This study differs from the previous two in the fact that they enrolled an asymptomatic population regardless of how patients achieved remission. The two studies recruiting patients with active UC detected no statistically significant difference between study arms in time to first relapse; however, it must be noted that in one of these studies, all the patients became steroid-free in the GMA group (9). 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). Despite our very promising results, these findings are limited by the amount of available data. More randomized controlled trials are necessary in this area to strengthen our results. This study has some potential limitations. 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**). Moreover, our funnel plots showed symmetry by visual assessment, but publication bias still cannot be ruled out because of the low number of included studies. Side-effects and safety data were not uniformly reported in most of the publications under analysis, according to the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines (15). Therefore, our second main objective, the safety assessment of GMA, was only achieved to a limited extent. Furthermore, this result is strongly limited by the high heterogeneity of studies. The most likely source of this is the heterogeneous nature of concomitant treatment. All in all, GMA seems to be a reasonable therapeutic option, but finding its exact place to treat UC demands further research. A particularly promising area could be remission maintenance.

6.1 Conclusion

Implications for practice: The results support the hypothesis that patients with active UC have a better chance of clinical remission if GMA is administered as an adjunctive therapy. As regards the frequency of AEs, we found no statistically significant difference between the two groups. With regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

Implications for research: Further studies are required to select patients who may benefit the most from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its role in remission induction. There is currently evidence available about induction and maintenance of clinical remission; however, the role of GMA concerning endoscopic and histological remission is currently unclear. If GMA is proven to be safe and effective, cost-effectiveness studies will also be worthwhile in the future.

7 Data availability statement

The data that support the findings of this study are available from the corresponding author, [A.H.], upon reasonable request.

8 Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

9 Author contributions

All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

S.K.: drafting the manuscript, selection of studies, data extraction, risk of bias assessment; D.N.: statistical analysis, preparation of the standardized data collection sheet, drafting the manuscript; P.H.: substantial contribution in study design, critical revision of the content; M.F.: selection of studies, data extraction, risk of bias assessment, drafting the manuscript; Z.S.: participation in the design of the study and its coordination, critical revision of the manuscript; B.E.: provided revisions to the scientific content of the manuscript, substantial contribution in design of the work; B.T.: substantial contribution in study design, drafting the manuscript; P.J.H.: preparation of the standardized data collection sheet, stylistic and grammatical revision of the manuscript, substantial contribution in study design; P.S.: expert in the field of gastroenterology, substantial contribution in study design and interpretation of data, preparation of study protocol and the first draft of the manuscript; A.H.: expert in the field of haematology, substantial contribution in study design and interpretation of data, preparation of study protocol and the first draft of the manuscript

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11 Conflict of interest

Authors do not have any conflicts of interest to declare.

12 Compliance with Ethical Standards

This study was prepared in accordance with the Committee on Publication Ethics (COPE) guidelines to respect third parties rights such as copyright and/or moral rights. Ethical approval was not required to conduct this project as data is not individualized and primary data was not collected.

13 Abbreviations

AE, adverse events; clinical activity index, CAI; confidence interval, CI; granulocyte and monocyte apheresis, GMA; inflammatory bowel disease, IBD; OR, odds ratio; RCT, randomized controlled trial; TSA, trial sequential analysis; UC, ulcerative colitis; weighted mean difference, WMD.

14 References

1. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. The Lancet. 2012;380(9853):1606-19.

2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis. The Lancet. 2017;389(10080):1756-70.

3. Saniabadi AR, Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H. Treating inflammatory bowel disease by adsorptive leucocytapheresis: a desire to treat without drugs. World J Gastroenterol. 2014;20(29):9699-715.

4. Matsumoto T, Fukunaga K, Kamikozuru K, Tozawa K, Yokoyama Y, Kusaka T, et al. Cytapheresis as a Non-Pharmacological Therapy for Inflammatory Bowel Disease. Transfus Med Hemother. 2008;35(1):18-23.

5. Yamamoto T. The role of granulocyte and monocyte apheresis in inflammatory bowel disease. J Crohns Colitis. 2013;7(3):e114.

6. Yamamoto T, Umegae S, Matsumoto K. Long-term clinical impact of early introduction of granulocyte and monocyte adsorptive apheresis in new onset, moderately active, extensive ulcerative colitis. J Crohns Colitis. 2012;6(7):750-5.

7. Sands BE, Sandborn WJ, Feagan B, Lofberg R, Hibi T, Wang T, et al. A randomized, double-blind, sham-controlled study of granulocyte/monocyte apheresis for active ulcerative colitis. Gastroenterology. 2008;135(2):400-9.

8. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Treatment of patients with acute ulcerative colitis: conventional corticosteroid therapy (MP) versus granulocytapheresis (GMA): a pilot study. Dig Liver Dis. 2007;39(5):430-4.

9. Fukunaga K, Yokoyama Y, Kamokozuru K, Nagase K, Nakamura S, Miwa H, et al. Adsorptive granulocyte/monocyte apheresis for the maintenance of remission in patients with ulcerative colitis: a prospective randomized, double blind, sham-controlled clinical trial. Gut Liver. 2012;6(4):427-33.

10. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

11. Hoboken. Cochrane Handbook for Systematic Reviews of Interventions: Wiley; 2008.

12. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

13. Cochran WG. The Combination of Estimates from Different Experiment. Biometrics. 1954;10:101-29.

14. Julian P T Higgins SGT, Jonathan J Deeks, Douglas G Altman. Measuring inconsistency in meta-analyses. British Medical Journal. 2003;327:557-60.

15. Bhatt A. International Council for Harmonisation E6(R2) addendum: Challenges of implementation. Perspectives in Clinical Research. 2017;8(4):162-6.

16. Schünemann H, Brożek J, Guyatt G, A O. GRADE handbook for grading quality of evidence and strength of recommendations: The GRADE Working Group, 2013; 2013.

17. Sawada K, Kusugami K, Suzuki Y, Bamba T, Munakata A, Hibi T, et al. Leukocytapheresis in ulcerative colitis: results of a multicenter double-blind prospective case-control study with sham apheresis as placebo treatment. Am J Gastroenterol. 2005;100(6):1362-9.

18. Bresci G, Parisi G, Mazzoni A, Scatena F, Capria A. Granulocytapheresis versus methylprednisolone in patients with acute ulcerative colitis: 12-month follow up. J Gastroenterol Hepatol. 2008;23(11):1678-82.

19. Domenech E, Panes J, Hinojosa J, Annese V, Magro F, Sturniolo GC, et al. Addition of Granulocyte/Monocyte Apheresis to Oral Prednisone for Steroid-dependent Ulcerative Colitis: A Randomized Multicentre Clinical Trial. J Crohns Colitis. 2018;12(6):687-94.

20. Eberhardson M, Karlen P, Linton L, Jones P, Lindberg A, Kostalla MJ, et al. Randomised, Double-blind, Placebo-controlled Trial of CCR9-targeted Leukapheresis Treatment of Ulcerative Colitis Patients. J Crohns Colitis. 2017;11(5):534-42.

21. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Kageoka M, et al. Intensive granulocyte and monocyte adsorption versus intravenous prednisolone in patients with severe ulcerative colitis: an unblinded randomised multi-centre controlled study. Dig Liver Dis. 2008;40(6):433-40.

22. Hanai H, Watanabe F, Yamada M, Sato Y, Takeuchi K, Iida T, et al. Adsorptive granulocyte and monocyte apheresis versus prednisolone in patients with corticosteroid-dependent moderately severe ulcerative colitis. Digestion. 2004;70(1):36-44.

23. Nakamura T, Kawagoe Y, Matsuda T, Ueda A, Ueda Y, Takahashi Y, et al. Effect of granulocyte and monocyte adsorption apheresis on urinary albumin excretion and plasma endothelin-1 concentration in patients with active ulcerative colitis. Blood Purif. 2004;22(6):499-504.

24. Emmrich J, Petermann S, Nowak D, Beutner I, Brock P, Klingel R, et al. Leukocytapheresis (LCAP) in the management of chronic active ulcerative colitis--results of a randomized pilot trial. Dig Dis Sci. 2007;52(9):2044-53.

25. Maiden L, Takeuchi K, Baur R, Bjarnason I, O'Donohue J, Forgacs I, et al. Selective white cell apheresis reduces relapse rates in patients with IBD at significant risk of clinical relapse. Inflamm Bowel Dis. 2008;14(10):1413-8.

26. Leukapheresis for inflammatory bowel disease: Interventional procedures guidance 2005 [Available from: https://www.nice.org.uk/guidance/ipg126.

27. Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, et al. Evidence-based clinical practice guidelines for inflammatory bowel disease. J Gastroenterol. 2018;53(3):305-53.

28. Shimoyama T, Kimura K, Hiwatashi N, Matsueda K, Munakata A, Asakura H, et al. Safety and Efficacy of Granulocyte and Monocyte Adsorption Apheresis in Patients With Active Ulcerative Colitis: A Multicenter Study. Journal of Clinical Apheresis. 2001;16:1-9.

29. Kanke K, Nakano M, Hiraishi H, Terano A. Clinical evaluation of granulocyte/monocyte apheresis therapy for active ulcerative colitis. Dig Liver Dis. 2004;36(12):811-7.

30. Kim HJ, Kim JS, Han DS, Yang SK, Hahm KB, Lee WI, et al. [Granulocyte and monocyte adsorption apheresis in Korean conventional treatment-refractory patients with active ulcerative colitis: a prospective open-label multicenter study]. The Korean journal of gastroenterology = Taehan Sohwagi Hakhoe chi. 2005;45(1):34-44.

31. Shimoyama T, Sawada K, Hiwatashi N, Sawada T, Matsueda K, Munakata A, et al. Safety and efficacy of granulocyte and monocyte adsorption apheresis in patients with active ulcerative colitis: a multicenter study. J Clin Apher. 2001;16(1):1-9.

32. Habermalz B, Sauerland S. Clinical effectiveness of selective granulocyte, monocyte adsorptive apheresis with the Adacolumn device in ulcerative colitis. Dig Dis Sci. 2010;55(5):1421-8.

33. Thanaraj S, Hamlin PJ, Ford AC. Systematic review: granulocyte/monocyte adsorptive apheresis for ulcerative colitis. Aliment Pharmacol Ther. 2010;32(11-12):1297-306.

34. Yoshino T, Nakase H, Minami N, Yamada S, Matsuura M, Yazumi S, et al. Efficacy and safety of granulocyte and monocyte adsorption apheresis for ulcerative colitis: a meta-analysis. Dig Liver Dis. 2014;46(3):219-26.

35. Imperiali G, Amato A, Terpin MM, Beverina I, Bortoli A, Devani M, et al. Granulocyte-Monocyte Apheresis in Steroid-Dependent, Azathioprine-Intolerant/Resistant Moderate Ulcerative Colitis: A Prospective Multicenter Study. Gastroenterol Res Pract. 2017;2017:9728324.

36. Yokoyama Y, Kawai M, Fukunaga K, Kamikozuru K, Nagase K, Nogami K, et al. Looking for predictive factors of clinical response to adsorptive granulocyte and monocyte apheresis in patients with ulcerative colitis: markers of response to GMA. BMC Gastroenterology. 2013;13(27).

37. Tanaka T, Sugiyama S, Goishi H, Kajihara T, Akagi M, Miura T. Treatment of children and adolescents with ulcerative colitis by adsorptive depletion of myeloid lineage leucocytes as monotherapy or in combination with low dose prednisolone after failure of first-line medications. BMC Gastroenterology. 2013;13(130).

38. Dignass AU, Eriksson A, Kilander A, Pukitis A, Rhodes JM, Vavricka S. Clinical trial: five or ten cycles of granulocyte-monocyte apheresis show equivalent efficacy and safety in ulcerative colitis. Aliment Pharmacol Ther. 2010;31(12):1286-95.

39. Ricart E, Esteve M, Andreu M, Casellas F, Monfort D, Sans M, et al. Evaluation of 5 versus 10 granulocyteaphaeresis sessions in steroid-dependent ulcerative colitis: A pilot, prospective, multicenter, randomized study. World Journal of Gastroenterology. 2007;13(15):2193-7.

40. Sakuraba A, Motoya S, Watanabe K, Nishishita M, Kanke K, Matsui T, et al. An open-label prospective randomized multicenter study shows very rapid remission of ulcerative colitis by intensive granulocyte and monocyte adsorptive apheresis as compared with routine weekly treatment. Am J Gastroenterol. 2009;104(12):2990-5.

41. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol. 2015;110(9):1324-38.

42. Yokoyama Y, Sawada K, Aoyama N, Yoshimura N, Sako M, Hirai F, et al. Efficacy of Granulocyte and Monocyte Adsorptive Apheresis in Patients With Inflammatory Bowel Disease Showing Lost Response to Infliximab. J Crohns Colitis. 2020;14(9):1264-73.

43. Nakamura M, Yamamura T, Maeda K, Sawada T, Mizutani Y, Ishikawa E, et al. Refractory Ulcerative Colitis Improved by Scheduled Combination Therapy of Vedolizumab and Granulocyte and Monocyte Adsorptive Apheresis. Internal medicine (Tokyo, Japan). 2020;59(23):3009-14.

44. Rodríguez-Lago I, Sempere L, Gutiérrez A, Núñez A, Leo Carnerero E, Hinojosa E, et al. Granulocyte-monocyte apheresis: an alternative combination therapy after loss of response to anti-TNF agents in ulcerative colitis. Scandinavian journal of gastroenterology. 2019;54(4):459-64.

15 Figures and tables

Figure 1: PRISMA flow chart representing the process of the study search and selection

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Table 1: Characteristics of included studies

Supplementary Figure 1: Risk of bias assessment on study level in studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 2: Risk of bias assessment across studies comparing patients with and without GMA as an adjunctive therapy

Supplementary Figure 3: Forest plot of studies comparing clinical remission induction or clinical improvement between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 4: Subgroup analysis of studies comparing clinical remission induction or clinical improvement after 12 weeks between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 5: Subgroup analysis based on criteria of remission in studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 6: Forest plot of studies comparing frequency of adverse events between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

Supplementary Figure 7: Results of Trial Sequential Analysis. A: clinical remission induction, B: clinical remission maintenance, C: Clinical remission induction based on remission criteria, D: Clinical remission induction or clinical improvement

Supplementary Table 1: List of reported adverse events.

Supplementary Table 2: Certainty of evidence by GRADE approach

Table 1: Characteristics of included studies

*** All patients received standard of care added to investigator/comparator. 1: one patient was excluded from analysis because of protocol deviations; 2: one patient was excluded from analysis because of protocol deviations; 3: one patient was excluded due to failure to return blood from the column; 4: minimization may be implemented without a random element, and this is considered to be equivalent to being random. Abbreviations: GMA=granulocyte/monocyte apheresis; n= number; CAI=Clinical Activity Index; EI=Endoscopic Index; 5-ASA=5-aminosalicylic acid; AZA=azathioprine; 6-MP=6-mercaptopurine;**

Clinical remission induction										
Study Name and Setting	**N⁰ of cycles (n)**	**Randomization***	**N⁰ of patients analyzed (n)**	**Patients achieving response**		**Patients achieving remission**		**Time of assessment**	**Outcome criteria**		**Concomitant medication**
				%	**n**	**%**	**n**		**Remission**	**Response**	
Bresci 2008 single center study	5	GMA	40	92.5	37	72.5	29	5 weeks	CAI<6; EI<4	CAI<6; EI>4	oral 5-ASA
		steroid	40	65.0	26	50.0	20				
Doménech 2018 multi-center study	7	GMA+steroid	62 [1]	58.1	36	19.4	12	12 weeks	Mayo ≤2 and no steroid use	Mayo score decrease ≥3 or at least 30% from baseline	stable dose AZA and steroid were allowed if started before randomization
		steroid	61 [2]	49.2	30	18.0	11				
Eberhardson 2017 single center study	5	GMA	14	57.1	8	35.7	5	12 days	Mayo score ≤3	Mayo score decrease ≥3 or at least 30% from baseline	stable dose of steroid; 5-ASA and/or thiopurines were allowed
		sham	8 [3]	37.5	3	12.5	1				
Hanai 2004 single center study	7	GMA	46	93.5	43	82.6	38	12 weeks	CAI≤4	CAI had fallen, but still 4<	steroids and/or 5-ASA
		steroid	23	78.3	18	65.2	15				
Hanai 2008 multi-center study	11	GMA	35	80.0	28	74.3	26	12 weeks	CAI≤4	CAI decreased by ≥5 points, but remained ≥5	all patients were on salicylates and the majority were on low dose steroid as well
		steroid	35	62.9	22	48.6	17				

Nakamura 2004 single center study	5	GMA	10	N/A	N/A	80.0	8	6 weeks	based on CAI, but not specified		all patients received steroid; 5-ASA was unchanged
		no GMA	10	N/A	N/A	20.0	2				
Sands 2008 A study multi-center study	10	GMA	31	67.7	21	16.1	5	12 weeks	Mayo score ≤2; 0-1 endoscopic score	Mayo score decrease ≥3	one or more of the following: 5-ASA agents, steroid, 6-MP or AZA
		sham	16	62.5	10	18.8	3				
Sands 2008 B study multi-center study	10	GMA	112	60.7	68	17.0	19	12 weeks	Mayo score ≤2; 0-1 endoscopic score	Mayo score decrease ≥3	one or more of the following: 5-ASA, steroid, 6-MP or AZA
		sham	56	50.0	28	10.7	6				
Sawada 2005[4] multi-center study	7	GMA	10	80.0	8	20.0	2	10 weeks	CAI=0	CAI improved >3	except for steroid, other medications remained unchanged
		sham	9	33.3	3	11.1	1				

Clinical remission maintenance

Study Name	Number of cycles (n)	Randomization	Number of patients analyzed (n)	Number of patients in clinical remission at the end of the study		Close-out examination	Outcome criteria for remission	Concomitant medication
				%	n			
Emmrich 2006 sigle center study	5	GMA	8	62.5	5	6 months	CAI≤4	all patients were on steroid; 5-ASA was allowed; AZA given at baseline remained unchanged
		no GMA	5	20.0	1			
Fukunaga 2012 single center study	12	GMA	10	40.0	4	12 months	CAI≤4	stable dose of AZA and steroids were allowed if started before randomization
		sham	11	9.1	1			
Maiden 2008 single center study	5	GMA	18	77.8	14	6 months	CAI≤6	only 5-ASA or oral steroid
		no GMA	19	26.3	5			

548

549

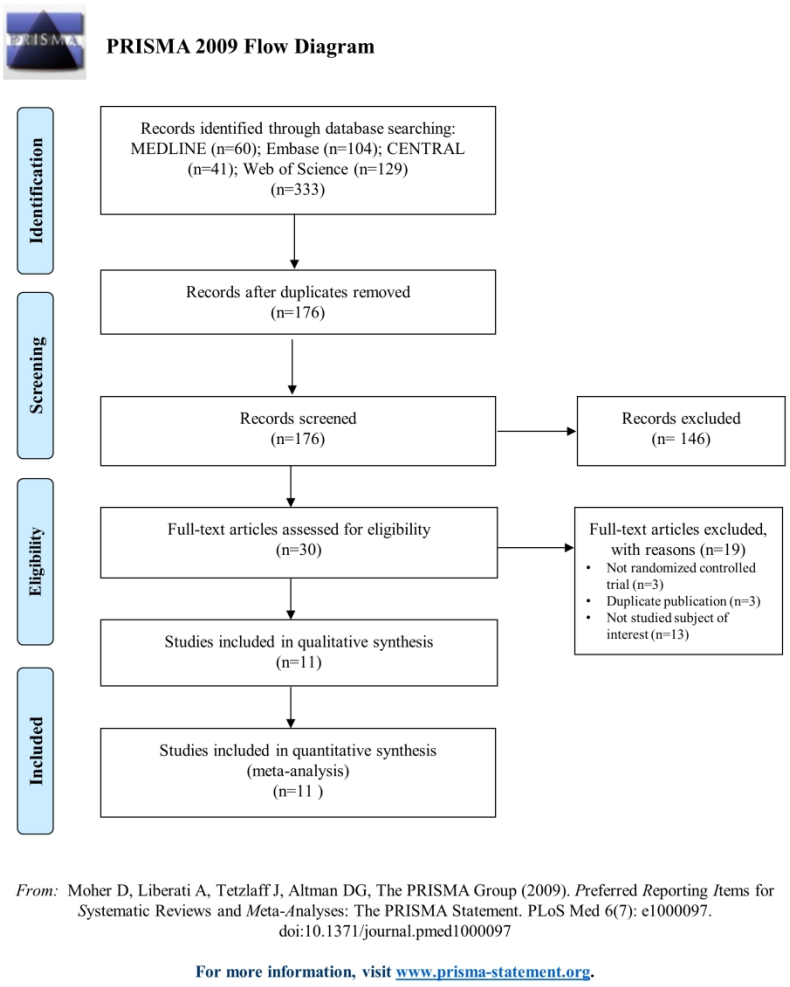

Figure 1: PRISMA flow chart representing the process of the study search and selection

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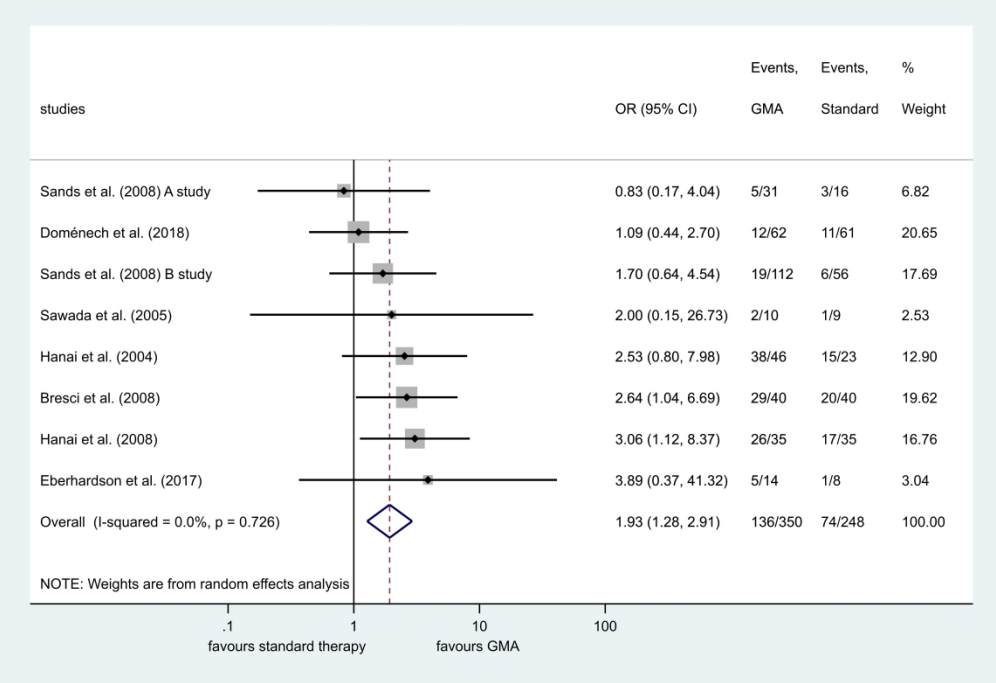

Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

272x186mm (600 x 600 DPI)

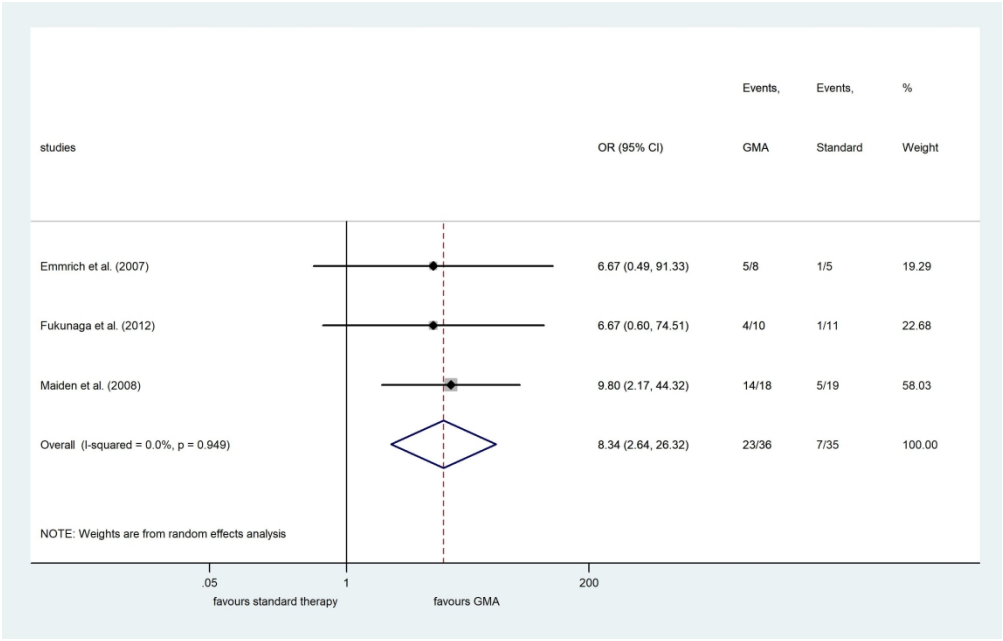

Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

137x87mm (600 x 600 DPI)

Supplemetary material

Granulocyte and monocyte apheresis as an adjunctive therapy to induce and maintain clinical remission in ulcerative colitis: A systematic review and meta-analysis

Szabolcs Kiss[1,2], Dávid Németh[2], Péter Hegyi[2,3], Mária Földi[1,2], Zsolt Szakács[2,3], Bálint Erőss[2,3], Benedek Tinusz[4], Péter Jenő Hegyi[2,5], Patrícia Sarlós[2,5], Hussain Alizadeh[1,6*]

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E-mail: alizadeh.hussain@pte.hu

Keywords: Inflammatory bowel disease

Search strategy for MEDLINE database

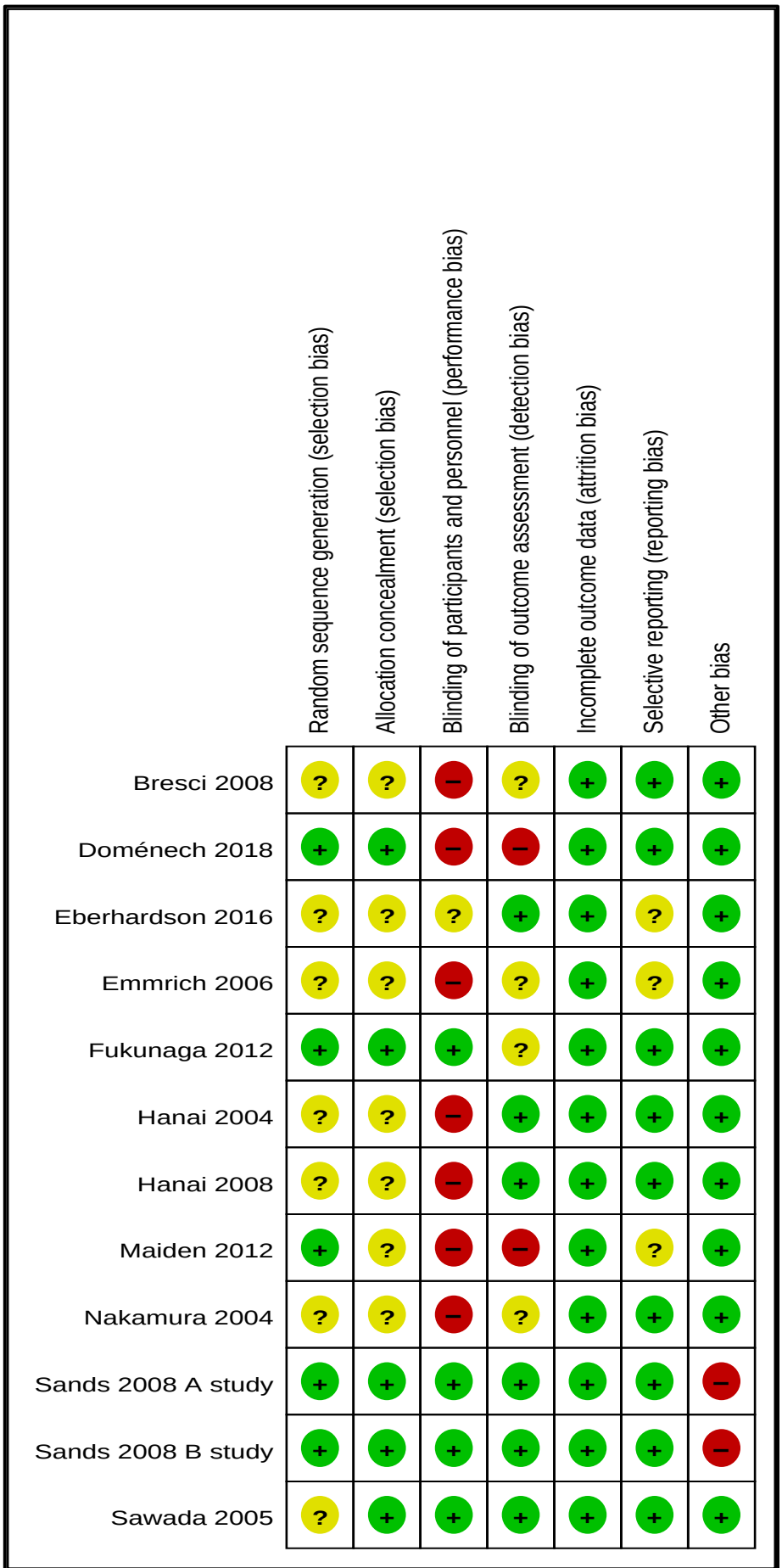
Date of search: 5th March, 2019

Full query: (gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND ("inflammatory bowel disease" OR "ulcerative colitis") AND (random*)

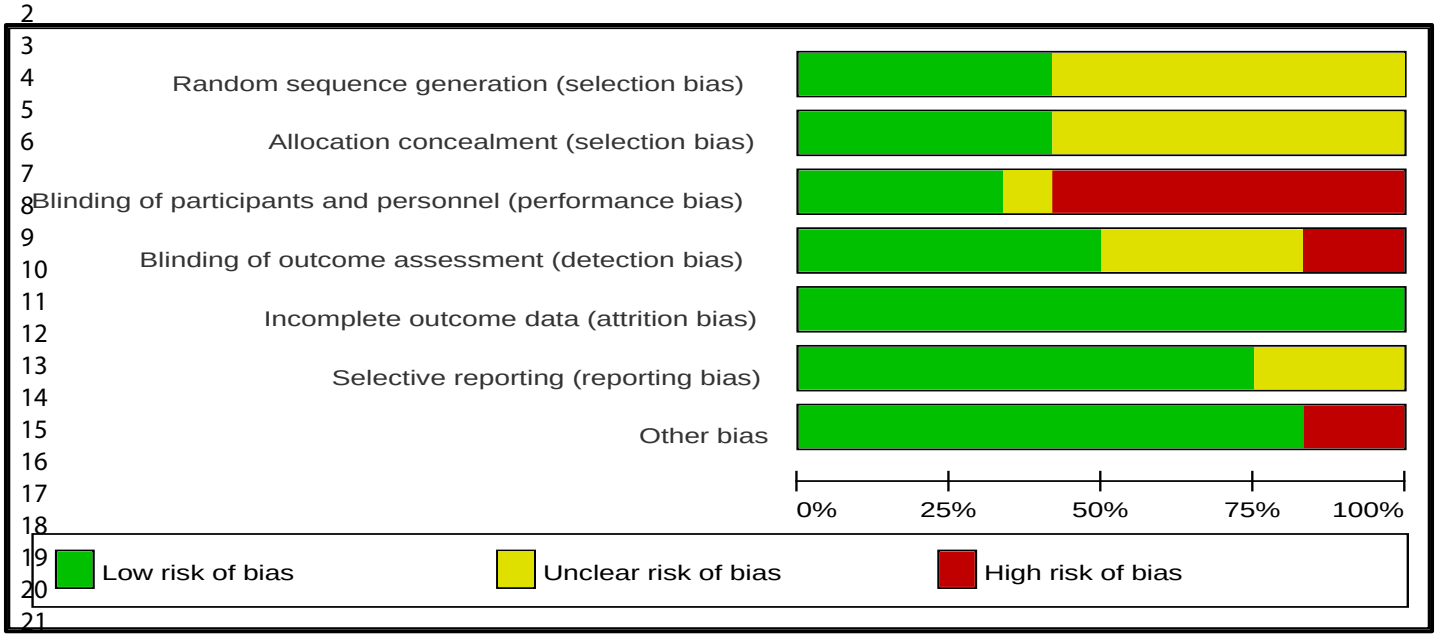
No filters or restrictions were applied.

Search	Query	Automatic explosion
#1	gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]
#2	"inflammatory bowel disease" OR "ulcerative colitis"	"inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields]
#3	random*	"random*"[All Fields]
#4	#1 AND #2	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]) AND ("inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields])
#5	#3 AND #4	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] AND "component"[All Fields] AND "removal"[All Fields]) OR "blood component removal"[All Fields] OR "apheresis"[All Fields]) OR ("adsorption"[MeSH Terms] OR "adsorption"[All Fields] OR "adsorptions"[All Fields] OR "adsorptive"[All Fields] OR "adsorptively"[All Fields] OR "adsorptives"[All Fields] OR "adsorptivities"[All Fields] OR "adsorptivity"[All Fields]) OR "cell separation"[All Fields] OR "leukapher*"[All Fields] OR "leukopher*"[All Fields] OR "leukocytapher*"[All Fields] OR "leukocytopher*"[All Fields] OR "lymphapher*"[All Fields] OR "lymphopher*"[All Fields] OR "lymphocytopher*"[All Fields] OR "lymphocytapher*"[All Fields]) AND ("inflammatory bowel disease"[All Fields] OR "ulcerative colitis"[All Fields]) AND "random*"[All Fields]

Supplementary Figure 1



Supplementary Figure 2



Detailed risk of bias assessment

Bresci et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomized study, but method was not specified in the manuscript
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant data have been reported. Adverse events were adequately reported.
Other bias	Low risk	The study appears to be free of other sources of bias.

Doménech et al. 2018	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...randomizaton codes were centerally generated using a computer procedure..." Blocked randomization was used.
Allocation concealment (selection bias)	Low risk	Quote: "...randomizaton codes were centerally generated using a computer procedure..."
Blinding of participants and personnel (performance bias)	High risk.	Open-label.
Blinding of outcome assessment (detection bias)	High risk	Quote: "...the endoscopist was not necessarily blinded..."
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat method was used. 123/125 patients completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported. Adequate

		description of adverse events.
Other bias	Low risk	The study appears to be free of other sources of bias.

Eberhardson et al. 2017	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Blocked randomization (3:2), but method is fully specified.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	Unclear risk	Double-blind, but insufficient data to permit judgement (form of placebo treatment was not described).
Blinding of outcome assessment (detection bias)	Low risk	Quote: "The FACS analysis was blinded to the clinical participants and the FACS analyst was also blinded before unblinding day 12."
Incomplete outcome data (attrition bias)	Low risk	1/9 patient from the placebo group was excluded from the study just after the randomization because of SADE (failure to return blood from the column). 2/14 (14%) were excluded from active study group because of adverse event and worsening of the disease, but analysis was conducted on full analyses set basis.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate.
Other bias	Low risk	The study appears to be free of other sources of bias.

Hanai et al. 2004	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized study, but method was not specified in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but other similar article from the authors was stated as unblinded.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Each patient was assessed blindly..."

Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Low risk	The study appears to be free of other sources of bias.

Hanai et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized study, but method is not described in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Stated as unblinded.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Each patient was assessed blindly..."
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	Low risk	The study appears to be free of other sources of bias.

Nakamura et al. 2004	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized, but the method was not specified in the manuscript
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	No information
Incomplete outcome data (attrition bias)	Low risk	60/66 completed the study; 1 took non-permitted drugs,1 relapsed just after the randomization, further 4 withdrew the consent.

Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	Low risk	The study appears to be free of other sources of bias.

Sands et al. 2008 A study	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "...using sealed envelopes with sequential numbers issued in blocks of 3..." and
Allocation concealment (selection bias)	Low risk	Quote: "...using sealed envelopes with sequential numbers issued in blocks of 3..." and
Blinding of participants and personnel (performance bias)	Low risk	Quote: "a polyvinylchloride bypass tube was inserted between the Adacolumn and the Adacircuit to permit bypass of the column among patients undergoing sham procedures."
Blinding of outcome assessment (detection bias)	Low risk	The gastroenterology team was blinded to the treatment assignment.
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat analysis; however, 66% of patients completed the study (6 patients left the study because of disease flare; 5 from apheresis group, 1 from sham group).
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	High risk	Quote: "Subjects who withdrew before the week 12 visit were treated as treatment failure for primary end point (clinical remission)." Comment: these imputation of ITT analysis may cause bias.

Sands et al. 2008 B study	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Randomization was performed according to a computer-generated scheme

		that used an integrated voice response system."
Allocation concealment (selection bias)	Low risk	Quote: "Randomization was performed according to a computer-generated scheme that used an integrated voice response system."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "a polyvinylchloride bypass tube was inserted between the Adacolumn and the Adacircuit to permit bypass of the column among patients undergoing sham procedures."
Blinding of outcome assessment (detection bias)	Low risk	The gastroenterology team was blinded to the treatment assignment.
Incomplete outcome data (attrition bias)	Low risk	Intention-to-treat analysis; however, 66% of patients completed the study (6 patients left the study because of disease flare; 5 from apheresis group, 1 from sham group).
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported
Other bias	High risk	Quote: "Subjects who withdrew before the week 12 visit were treated as treatment failure for primary end point (clinical remission)." Comment: these imputation of ITT analysis may cause bias.

Sawada et al. 2005	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	minimization by an independent controller.
Allocation concealment (selection bias)	Unclear risk	Quote: “The assignment of the enrolled patients to the active group or the sham group was performed by a controller who was independent of the other staff, patients, and relatives.”
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Both columns were covered with an opaque material so that they could

		not be distinguished by the patients."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "To ensure proper blinding within the clinical evaluation, the medical staffs of each institution were separated into two independent groups."
Incomplete outcome data (attrition bias)	Low risk	All of the enrolled eligible patients were evaluated.
Selective reporting (reporting bias)	Low risk	All outcomes of interest were reported.
Other bias	Low risk	The study appears to be free of other sources of bias. Comment: these imputation of ITT analysis may cause bias.

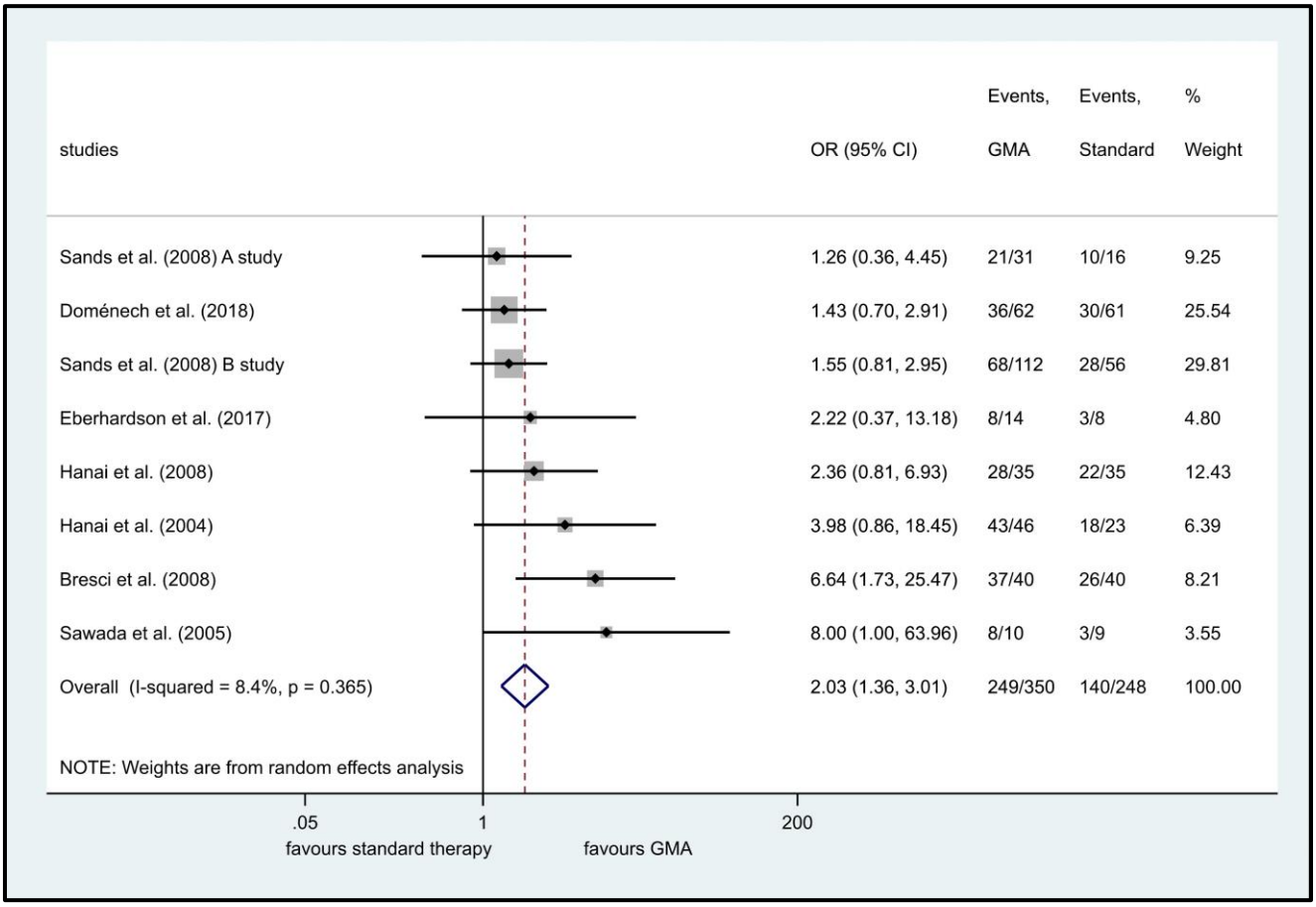
Emmrich et al. 2006	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomized, but method is not specified in the manuscript.
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Not described in the manuscript, but probably not done, because the trial compared an interventional procedure to drug treatment only.
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	Only 1/9 patient from active group discontinued the study.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate.
Other bias	Low risk	The study appears to be free of other sources of bias.

Fukunaga et al. 2012	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Blocked randomization according to a computer-generated scheme.
Allocation concealment (selection bias)	Low risk	Patients were randomized in a 1:1:1 ratio by a statistician at an independent organization.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Both patients and the physician were blinded by a curtain."

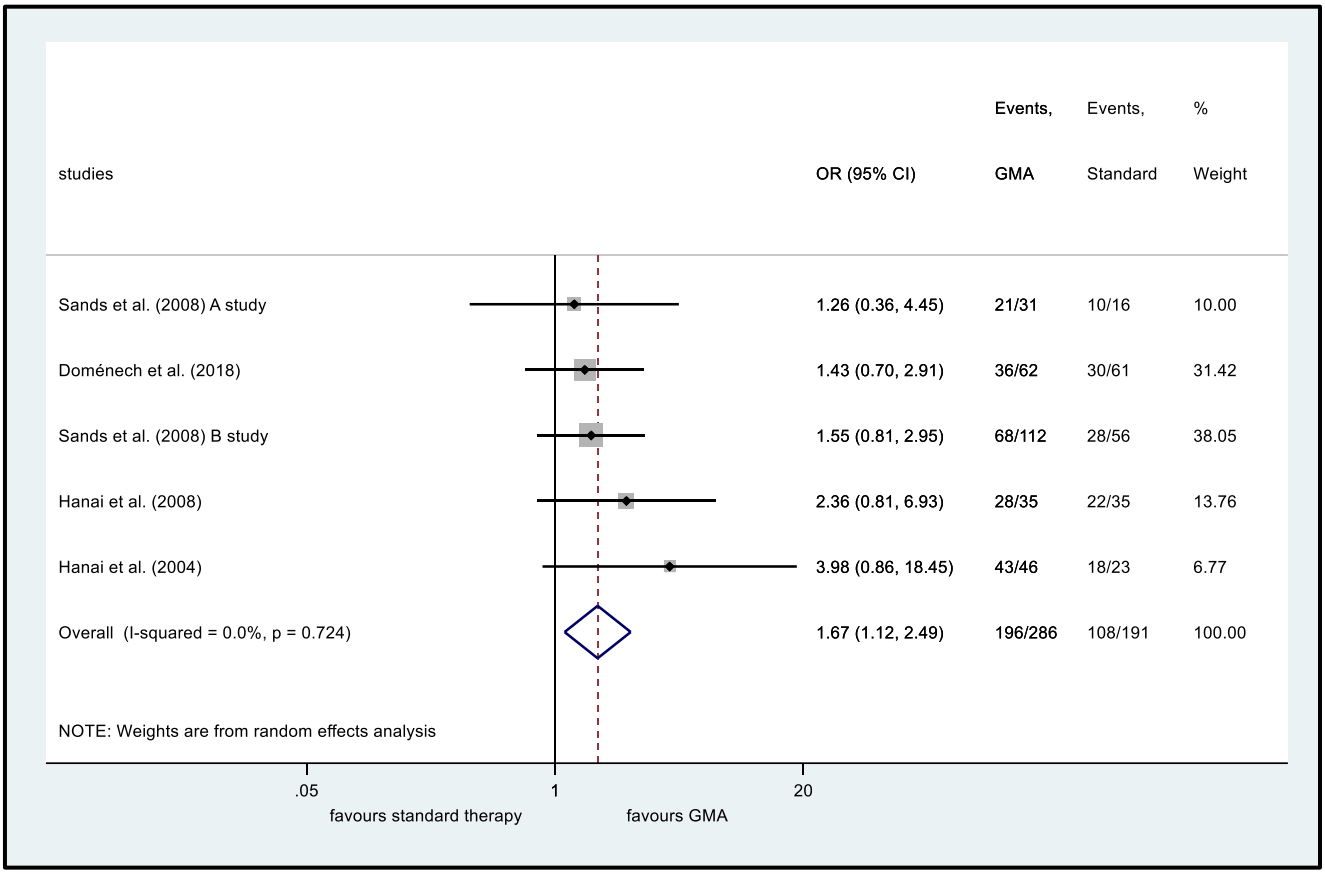
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	21/22 completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Unclear risk	Concomitant therapeutic regimen was not described clearly, and the authors stated: "a significant fraction of patients in each arm were on concomitant PSL or AZA and this enabled us to assess the contribution of these medications"

Maiden et al. 2008	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Randomization was conducted using a linear random number generator of 0 to 1."
Allocation concealment (selection bias)	Unclear risk	Not described in the manuscript.
Blinding of participants and personnel (performance bias)	High risk	Open label
Blinding of outcome assessment (detection bias)	High risk	Open label
Incomplete outcome data (attrition bias)	Low risk	Number of patients at baseline and at the end of the follow-up are the same.
Selective reporting (reporting bias)	Unclear risk	Report of adverse events seems to be inadequate. Number of events in the control group was not described.
Other bias	Low risk	The study appears to be free of other sources of bias.

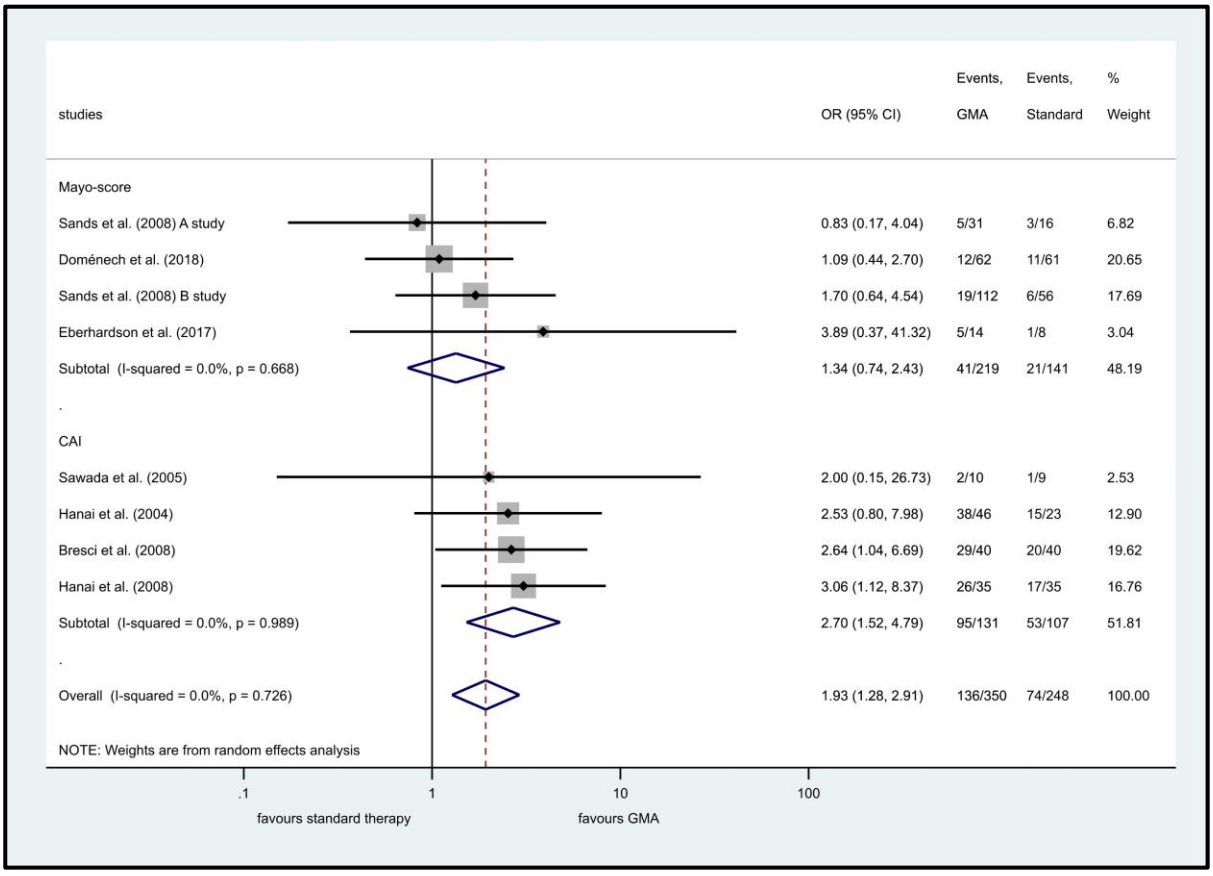
Supplementary Figure 3



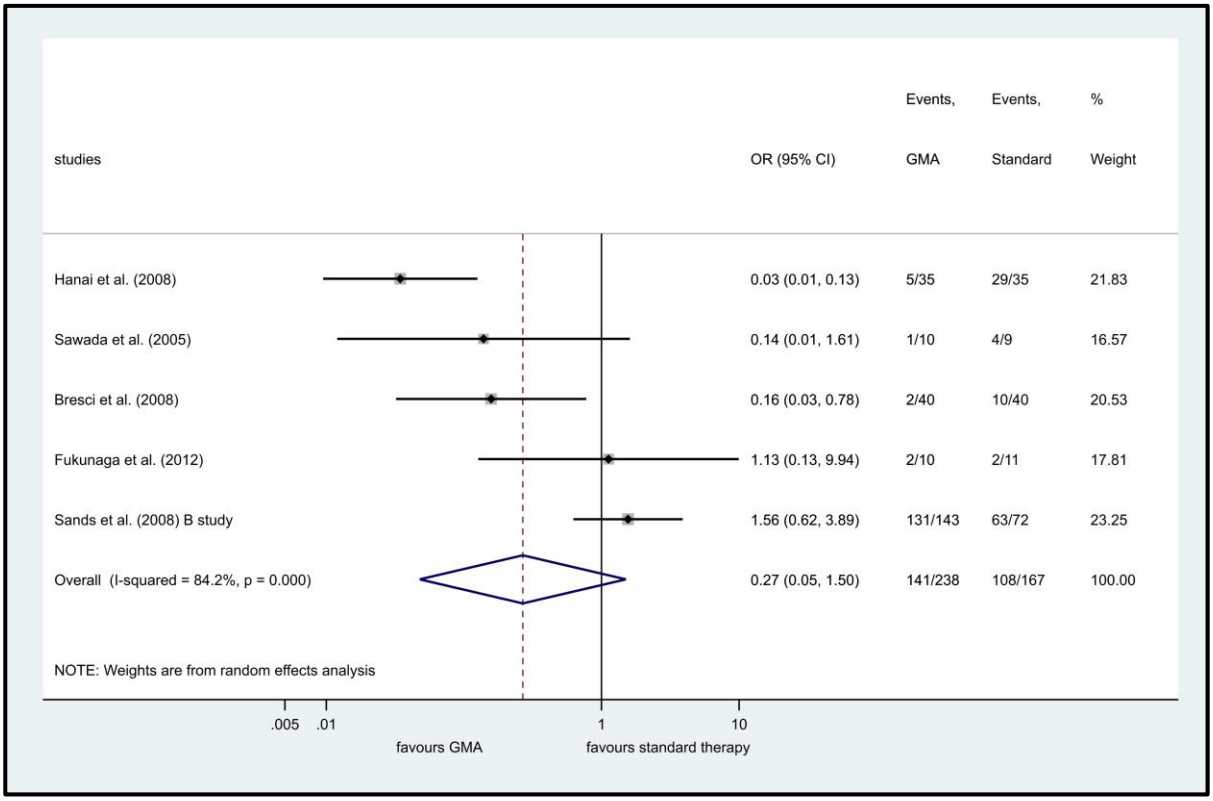
Supplementary Figure 4



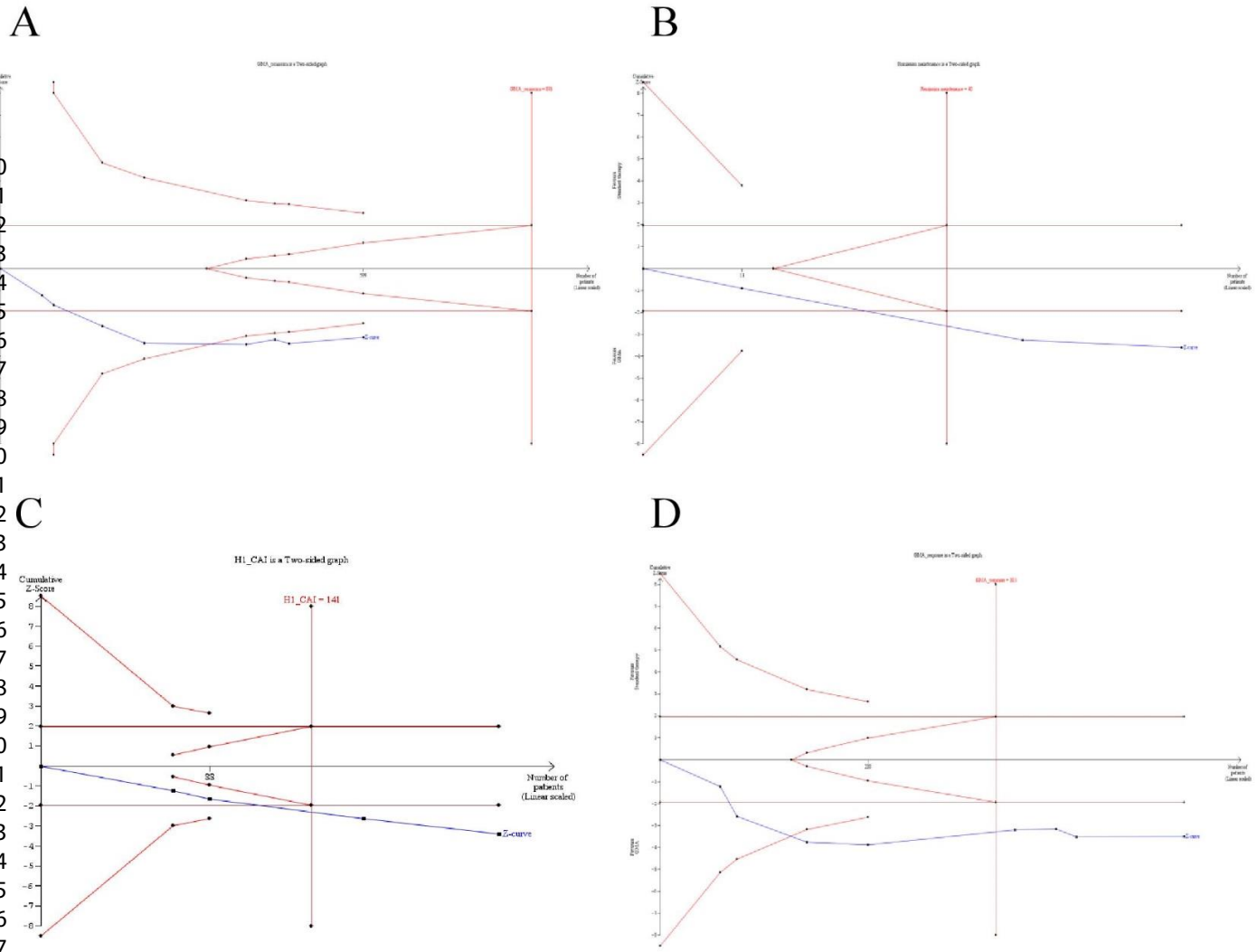
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Table 1

Study	Reported adverse events
Hanai et al. 2004	flushing, nausea, mild fever
Sawada et al. 2005	fever, skin rash, back pain
Bresci et al. 2008	headache, gastrointestinal intolerance, facies lunaris, vascular hypertension, glucose intolerance
Fukunaga et al. 2012	nausea, skin itchiness
Sands et al. 2008	headache, disease flare-up, decreased diastolic blood pressure, nasopharyngitis, hypotension, nausea, fatigue, post procedure hematoma, abdominal pain, dizziness, vomiting, vessel puncture site bruise, diarrhea, upper respiratory tract infection, flatulence

Supplementary Table 2

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	standard therapy for clinical remission induction and GMA as an adjunctive therapy	standard therapy for clinical remission induction	Relative (95% CI)	Absolute (95% CI)		
Clinical remission rate (assessed with: CAI or Mayo-score)												
8	randomized trials	very serious	not serious	not serious	serious	none	136/350 (38.9%)	74/249 (29.7%)	**OR 1.94** (1.28 to 2.92)	**153 more per 1 000** (from 54 more to 255 more)	⊕◯◯◯ VERY LOW	CRITICAL
Clinical response and clinical improvement (CAI or Mayo-score)												
8	randomized trials	very serious	not serious	not serious	not serious	none	249/350 (71.1%)	140/249 (56.2%)	**OR 2.05** (1.37 to 3.06)	**162 more per 1 000** (from 75 more to 235 more)	⊕⊕◯◯ LOW	CRITICAL
Clinical remission maintenance rate (assessed with: CAI)												
3	randomized trials	serious	not serious	serious [a]	not serious	none	39/36 (108.3%)	17/35 (48.6%)	**OR 8.34** (2.64 to 26.32)	**402 more per 1 000** (from 228 more to 476 more)	⊕⊕◯◯ LOW	CRITICAL
Adverse events												
5	randomized trials	very serious	not serious	very serious [b]	very serious [c,d]	publication bias strongly suspected	141/238 (59.2%)	108/167 (64.7%)	**OR 0.27** (0.05 to 1.50)	**316 fewer per 1 000** (from 563 fewer to 86 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Steroid-sparing effect												
3	randomized trials	serious	not serious	not serious	very serious [d]	none	66	43	-	WMD **6.83 mg/day lower** (14.47 lower to 0.81 higher)	⊕◯◯◯ VERY LOW	IMPORTANT

CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Duration of follow-up differs among studies (6 months or 12 months). b. Pool of adverse events differs among studies. c. The optimal information size criterion is not met. d. TSA could not be carried out.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2, 3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3, 4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Suppl.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4, 5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5, 6, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6, Suppl. Figure 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6, Figure 2-3, Suppl. Figure 4-5
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency	9, Figure 2-3, Suppl. Figure 3, Suppl. Figure 6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Suppl. Figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Suppl. Figure 4-5; Suppl Figure 7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Suppl. Table 1

PRISMA 2009 Checklist

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	2, 9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

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