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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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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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15 **Keywords:** Inflammatory bowel disease; IMMUNOLOGY; Gastroenterology;
16 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 (1) and steroid-sparing effect. Odds ratios (OR) with 95% confidence intervals were calculated. Trial Sequential Analyses (TSA) were performed to adjust 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.

Word count: 3801

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*

90 OR lymphopher* OR lymphocytopher* OR lymphocytapher*) AND (“inflammatory bowel disease”
91 OR “ulcerative colitis”) AND (random*).

4.2 Eligibility criteria

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

96 Specific criteria for clinical remission induction: patients with active UC (*Population*₁),
97 standard therapy for remission induction and GMA as an adjunctive therapy (*Intervention*₁), and
98 standard therapy for remission induction (*Comparison*₁); *Outcomes*₁: clinical response rate and clinical
99 remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.[12, 13]

100 Specific criteria for clinical remission maintenance: patients with UC in clinical remission
101 induced by GMA (*Population*₂), standard therapy for remission maintenance and GMA as an
102 adjunctive therapy (*Intervention*₂), and standard therapy for remission maintenance (*Comparison*₂);
103 *Outcomes*₂: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

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

4.3 Data extraction

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

4.4 Risk of bias assessment

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

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

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2 130 7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials,
3 131 and imputation of intention-to-treat (ITT) analysis)
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5 132 4.5 Statistical analysis 6

7 133 The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR)
8 134 with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered
9 135 statistically significant (except for heterogeneity, for which a p value <0.10 was considered
10 136 significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR,
11 137 WMD, and weights are presented in forest plots. The random-effects model was used to pool effect
12 138 sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating
13 139 Higgins' I² indicator.[(13, 14)6] The Q statistics were computed as the squared deviations from the
14 140 pooled effect of the weighted sum of individual study effects, with the weights being used in the
15 141 pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees
16 142 of freedom (where k was the number of studies). The I² index corresponds to the percentage of the total
17 143 variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane
18 144 Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–
19 145 60%), substantial (50–90%), and considerable (75–100%).[(11)] Subgroup analysis was performed as
20 146 described in the study protocol if a sufficient number of studies was available. Funnel plots were used
21 147 to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed
22 148 for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal
23 149 information size (OIS). This methodology combines an information size with the threshold of statistical
24 150 significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version
25 151 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).
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30 152 4.6 Quality of evidence 31

32 153 The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality
33 154 of evidence for each outcome (15, 16). Disagreements were resolved by consensus.
34

35 155 5 Results 36

37 156 5.1 Search and selection 38

39 157 The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After
40 158 screening and assessment for eligibility, eleven full-text articles containing one minimized controlled
41 159 trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active
42 160 UC, and three studies contained data on patients with UC in clinical remission.
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44

45 161 5.2 Characteristics of the studies included 46

47 162 The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission
48 163 induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598
49 164 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients
50 165 received GMA, and 248 were in control groups. All the participants had active UC and were treated
51 166 with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received
52 167 standard of care added to the intervention/comparator.
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54 168 Both GMA and control were added to conventional treatment. In terms of main outcomes, the studies
55 169 investigated the rate of clinical remission and clinical response. Investigators assessed the activity of
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2 170 UC with either the Mayo score or CAI. One study required steroid-free remission to regard cases as
3 171 being in clinical remission.

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5 172 In the case of clinical remission maintenance, all the studies were randomized controlled trials. A total
6 173 of 71 participants (mean: 24, ranging from 13 to 37) were included in this meta-analysis: 36 patients
7 174 received GMA, and 35 were in control groups. All the participants had ulcerative colitis in remission
8 175 and were treated with Adacolumn® or Cellisorba®. One trial evaluated GMA vs sham control (24) and
9 176 two trials assessed GMA compared to standard therapy alone (9, 25). Both GMA and sham control
10 177 were added to conventional treatment. In terms of main outcome, the studies investigated the rate of
11 178 clinical relapse.

12
13 179 Three studies also reported on the steroid-sparing effect of GMA (9, 17, 22).

14 15 180 **5.3 Risk of bias assessment**

16
17 181 A summary of risk of bias assessment is shown in **Supplementary Figure 1 and Supplementary**
18 182 **Figure 2**. One study was graded at a high risk of selection bias because it used minimization for
19 183 sequence generation (17). Three unblinded studies were at high risk of performance bias (19, 22, 25).
20 184 Because of the nature of the intervention, four studies which lacked a description of the blinding
21 185 process were interpreted as having a high risk of bias (18, 21, 23, 24). As regards assessment blinding,
22 186 two unblinded studies were judged to be at high risk of bias (19, 25). Two studies were deemed as
23 187 having a high risk of other bias; although they used ITT analysis, they considered subjects who left the
24 188 study as a treatment failure that may lead to bias (7).

25 26 27 189 **5.4 Efficacy and safety of GMA in clinical remission induction**

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29 190 Seven randomized and one minimized controlled trial evaluated clinical remission induction. GMA
30 191 therapy was associated with a better clinical response rate compared to the control group (OR = 2.03,
31 192 95% CI = 1.36–3.01, $p < 0.001$, $I^2 = 8.4\%$) (**Supplementary Figure 3**). Patients undergoing GMA
32 193 therapy had a higher remission rate compared to standard therapy without GMA (OR = 1.93, 95% CI
33 194 = 1.28–2.91, $p = 0.002$, $I^2 = 0.0\%$) (**Figure 2**). Sub-group analyses were performed based on activity
34 195 indices and number of GMA cycles. No difference was found between the two groups in studies
35 196 assessing UC with the Mayo score (OR = 1.34, 95% CI = 0.74–2.43, $p = 0.334$, $I^2 = 0.0\%$), but the
36 197 remission induction was more successful in studies using CAI for assessment (OR = 2.70, 95% CI =
37 198 1.52–4.79, $p = 0.001$, $I^2 = 0.0\%$) (**Supplementary Figure 4**). A significant difference was found in
38 199 studies using five cycles compared to the control (OR = 2.78, 95% CI = 1.17–6.60, $p = 0.021$, $I^2 = 0.0\%$)
39 200 and more than five cycles compared to standard therapy alone (OR = 1.73, 95% CI = 1.08–2.77,
40 201 $p = 0.022$, $I^2 = 0.0\%$). There was no statistically significant difference in the number of AEs ($p = 0.135$)
41 202 (**Supplementary Figure 5**). No statistically significant steroid-sparing effect was detected among
42 203 patients with active UC ($p = 0.080$). A list of reported AEs is presented in **Supplementary Table 1**.

43 44 45 46 204 **5.5 Efficacy and safety of GMA in clinical remission maintenance**

47
48 205 Three randomized clinical trials evaluated the clinical remission rate in remitting UC induced by GMA.
49 206 Patients receiving GMA had a higher rate of clinical remission maintenance (OR = 8.34, 95% CI =
50 207 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
51 208 in this population.

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2 2093
4 210 **5.6 Trial Sequential Analysis**

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6 211 Based on a TSA, the cumulative Z curve crossed the trial sequential significance boundary as regards
7 212 clinical remission induction and clinical remission maintenance (power=80.0%; alpha=5.0%)
8 213 (**Supplementary Figure 6**). Moreover, clinical remission maintenance exceeded the required meta-
9 214 analysis sample size, possibly suggesting that further clinical trials are not required. A TSA for AEs
10 215 and steroid-sparing effects could not be carried out due to insufficient information size.

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13 216 **5.7 Quality of evidence**

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15 217 The GRADE analysis rated the quality of evidence for primary and secondary outcomes at a very low
16 218 to moderate level. GRADE evidence profile is shown in **Supplementary Table 2**.

17
18 219 **6 Discussion**

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20 220 The main goal of care is to achieve and maintain remission of UC. This condition is usually treated by
21 221 a step-up approach, during which treatments are switched or additional treatment is administered to
22 222 optimize current therapy. There are several therapeutic agents to slow down the clinical activity of UC.
23 223 Corticosteroids, 5-aminosalicylates, immunosuppressive agents, and tumour necrosis alpha-inhibitors
24 224 are commonly used, and new therapeutic targets, such as anti-adhesion molecules and anti-interleukins,
25 225 are emerging. Despite these multiple therapeutic options, there is still a need to expand the scope of
26 226 treatment methods due to possible development of intolerance or resistance to current treatments. After
27 227 running out of treatment options, surgical therapy is frequently the last remaining option for patients.
28 228 GMA is a novel non-pharmacologic treatment option for active and remitting UC, by which activated
29 229 granulocytes and monocytes are removed from the circulation. These cells may contribute to the
30 230 pathogenesis of UC.

31
32 231 Guidelines describing the role of GMA in UC are in agreement on the potential beneficial effect and
33 232 favourable safety profile. They also agree that there is insufficient evidence in this field of practice (26,
34 233 27).

35
36 234 To our knowledge, the first report on the efficacy of GMA in UC was published in Japan in 2001 (28).
37 235 This study found a considerably high remission rate with only five sessions of GMA in patients
38 236 refractory to conventional drug therapy. Subsequent studies from the early 21st century had similar
39 237 results (29-31). In 2008, Sands et al. failed to prove a significant difference in clinical remission rate
40 238 between GMA and a placebo on a relatively large population (7). However, this study was not free of
41 239 attrition bias; a high proportion of patients were lost to follow-up. Three systematic reviews and meta-
42 240 analyses have been conducted in this field so far (32-34). All of them have agreed on the benefit of
43 241 GMA in clinical remission induction, and they pointed out the necessity for more trials with a rigorous
44 242 and clear design to further narrow the focus on specific patient groups. These studies used one to three
45 243 databases for a systematic search and selection.

46
47 244 In our current meta-analysis, a broader literature search was carried out, and the role of GMA in clinical
48 245 remission maintenance was assessed. Our work supported the hypothesis that GMA improves the rates
49 246 of clinical response and clinical remission in patients with UC. It should be noted that response and
50 247 remission rates defined by symptom scores should be cautiously interpreted because they also include
51 248 subjective elements, such as overall physician judgement on disease activity. A few recent
52 249 retrospective and prospective studies have suggested certain prognostic factors in the therapeutic
53 250 response (35-37). It seems that younger patients respond better to GMA therapy, whereas gender and
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2 251 smoking status showed no difference in response to treatment (35). Yokoyama et al. found that shorter
3 252 duration of UC and lower cumulative corticosteroid dose are associated with a higher efficacy rate
4 253 (36). In their study, patients who received GMA treatment immediately after relapse were the best
5 254 responders. It would be advisable to conduct further research to identify subgroups of UC where
6 255 patients benefit the most from GMA (38).

8 256 To date, there is no uniformly accepted GMA regimen. There are RCTs to compare a ten-cycle and a
9 257 five-cycle GMA regimen. Dignass et al. and Ricart et al. found similar remission rates between ten and
10 258 five cycles (46% vs. 36%, $p=0.479$; 35.7% vs 45.5%, $p>0.05$, respectively) (38, 39). The latter study
11 259 also showed a steroid-sparing effect in the group receiving ten cycles of GMA. Sakuraba et al. found
12 260 that an improved remission rate is associated with intensive GMA (54.0% vs 71.2%, $p=0.029$ in five-
13 261 and ten-cycle regimens, respectively) (40). In our meta-analysis, the number of GMA cycles varied
14 262 among studies as well. We assessed the efficacy of GMA based on the two main regimens in previous
15 263 trials. Both groups showed a benefit of adding GMA to the therapy compared to standard treatment
16 264 alone.

19 265 We found no significant difference between the two groups as regards AEs. Further studies are called
20 266 for to provide a higher level of evidence on this topic. They would be particularly important for specific
21 267 subgroups where the safety profile is of paramount importance, such as in cytomegalovirus infection,
22 268 adolescence, and pregnancy. Clinical trials should also target these populations because fewer
23 269 therapeutic options are available for them and the safety profile of GMA seems favourable compared
24 270 to other treatments.

26 271 As with any therapeutic option, cost-effectiveness should also be considered. The cost of GMA is much
27 272 higher compared to regular medication, such as corticosteroids, but GMA could be cost-effective in
28 273 the long term. The use of GMA may reduce the cost of medical services, hospitalization, and surgery
29 274 in the long term. Nevertheless, GMA's safety profile is in sharp contrast to multiple severe AEs
30 275 associated with conventional pharmacologics and biologics.

32 276 To our knowledge, this is the first meta-analysis to assess the role of GMA in UC remission
33 277 maintenance. Our study showed that the addition of GMA enhances the proportion of patients who can
34 278 maintain their remission. Fukunaga et al. and Emmrich et al. enrolled clinically active UC patients
35 279 based on CAI (9, 24). After successful induction therapy with the inclusion of GMA, patients achieving
36 280 clinical remission were allocated to groups with and without GMA treatment for remission
37 281 maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin, which is
38 282 considered as a risk factor of relapse (25). This study differs from the previous two in the fact that they
39 283 enrolled an asymptomatic population regardless of how patients achieved remission. The two studies
40 284 recruiting patients with active UC detected no statistically significant difference between study arms
41 285 in time to first relapse; however, it must be noted that in one of these studies, all the patients became
42 286 steroid-free in the GMA group (9). Maiden et al. found that time to first relapse was significantly higher
43 287 in patients receiving GMA (99 ± 73 days vs. 161 ± 44 days, $p=0.0004$). Despite our very promising
44 288 results, these findings are limited by the amount of available data. More randomized controlled trials
45 289 are necessary in this area to strengthen our results. This study has some potential limitations. Allowed
46 290 concomitant therapies have differed among included studies; therefore, our estimates may have been
47 291 subject to bias, as reflected by the grade of evidence (**Supplementary Table 2**). Moreover, our funnel
48 292 plots showed symmetry by visual assessment, but publication bias still cannot be ruled out because of
49 293 the low number of included studies. Side-effects and safety data were not uniformly reported in most
50 294 of the publications under analysis, according to the International Conference on Harmonisation-Good
51 295 Clinical Practice (ICH-GCP) guidelines (41). Furthermore, this result is strongly limited by the high
52 296 heterogeneity of studies. All in all, GMA seems to be a reasonable therapeutic option, but finding its

2 297 exact place to treat UC demands further research. A particularly promising area could be remission
3 298 maintenance.

5 299 **6.1 Conclusion**

7 300 Implications for practice: The results support the hypothesis that patients with active UC have a
8 301 better chance of remission if GMA is administered as an adjunctive therapy. As regards the
9 302 frequency of AEs, we found no statistically significant difference between the two groups. With
10 303 regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

13 304 Implications for research: Further studies are required to select patients who may benefit the most
14 305 from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its
15 306 role in remission induction. If GMA is proven to be safe and effective, cost-effectiveness studies will
16 307 also be worthwhile in the future.

18 308 **7 Data availability statement**

20 309 The data that support the findings of this study are available from the corresponding author, [A.H.],
22 310 upon reasonable request.

24 311 **8 Patient and Public Involvement**

26 312 It was not appropriate or possible to involve patients or the public in the design, or conduct, or
27 313 reporting, or dissemination plans of our research.

29 314 **9 Author contributions**

31 315 S.K.: preparation of the draft of the manuscript, selection of studies, data extraction, risk of bias
32 316 assessment; G.N.: statistical analysis, preparation of the standardized data collection sheet; P.H.:
33 317 substantial contribution in study design; M.F.: selection of studies, data extraction; F.D.: selection of
34 318 studies, data extraction; B.N. data extraction, risk of bias assessment; M.F.J.: preparation of the
35 319 standardized data collection sheet, stylistic and grammatical revision of the manuscript; K.O.: risk of
36 320 bias assessment, stylistic and grammatical revision of the manuscript; Z.M.: expert in the field of
37 321 anaesthesiology and intensive therapy, substantial contribution in study design and interpretation of
38 322 data, preparation of the manuscript; N.Z.: substantial contribution in study design; A.P.: preparation
39 323 of the study protocol; P.J.H.: preparation of the standardized data collection sheet, stylistic and
40 324 grammatical revision of the manuscript; Z.S.: participation in the design of the study and its
41 325 coordination; P.G.: provided revisions to the scientific content of the manuscript; B.E.: provided
42 326 revisions to the scientific content of the manuscript; A.H.: expert in the field of haematology,
43 327 substantial contribution in study design and interpretation of data, preparation of study protocol and
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55 334 design, data collection, analysis, interpretation, or preparation of the manuscript.

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.

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8 461 **15 Figures and tables**

9
10 462 **Figure 1:** PRISMA flow chart representing the process of the study search and selection

11
12 463 **Figure 2:** Forest plot of studies comparing clinical remission induction between patients with and
13 464 without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and
14 465 vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on
15 466 the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of
16 467 the diamonds represent the CIs.

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19 468 **Figure 3:** Forest plot of studies comparing clinical remission maintenance between patients with and
20 469 without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and
21 470 vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on
22 471 the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of
23 472 the diamonds represent the CIs.

25 473 **Table 1:** Characteristics of included studies

27 474 **Supplementary Figure 1:** Risk of bias assessment on study level in studies comparing patients with
28 475 and without GMA as an adjunctive therapy

31 476 **Supplementary Figure 2:** Risk of bias assessment across studies comparing patients with and
32 477 without GMA as an adjunctive therapy

34 478 **Supplementary Figure 3:** Forest plot of studies comparing clinical remission induction or clinical
35 479 improvement between patients with and without GMA as adjunctive therapy. Black diamonds
36 480 represent the individual studies effect and vertical lines show the corresponding 95% confidence
37 481 intervals (8). Size of the grey squares reflect on the weight of a particular study. The blue diamond
38 482 the overall or summary effect. The outer edges of the diamonds represent the CIs.

41 483 **Supplementary Figure 4:** Subgroup analysis based on criteria of remission in studies comparing
42 484 clinical remission induction between patients with and without GMA as adjunctive therapy. Black
43 485 diamonds represent the individual studies effect and vertical lines show the corresponding 95%
44 486 confidence intervals. Size of the grey squares reflect on the weight of a particular study. The blue
45 487 diamond the overall or summary effect. The outer edges of the diamonds represent the CIs.

48 488 **Supplementary Figure 5:** Forest plot of studies comparing frequency of adverse events between
49 489 patients with and without GMA as adjunctive therapy. Black diamonds represent the individual
50 490 studies effect and vertical lines show the corresponding 95% confidence intervals. Size of the grey
51 491 squares reflect on the weight of a particular study. The blue diamond the overall or summary effect.
52 492 The outer edges of the diamonds represent the CIs.

54 493 **Supplementary Figure 6:** Results of Trial Sequential Analysis. A: clinical remission induction, B:
55 494 clinical remission maintenance, C: Clinical remission induction based on remission criteria, D:
56 495 Clinical remission induction or clinical improvement

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2 496 **Supplementary Table 1:** List of reported adverse events.

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4 497 **Supplementary Table 2:** Certainty of evidence by GRADE approach

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498 **Table 1: Characteristics of included studies**

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

Clinical remission induction											
Study Name	Country / Setting	N ^o of cycles (n)	Randomization*	N ^o of patients analyzed (n)	Patients achieving Res		Patients achieving Rem		Time of assessment	Outcome criteria (Rem and Res)	Concomitant medication
					%	n	%	n			
Brescia 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			

GMA in ulcerative colitis

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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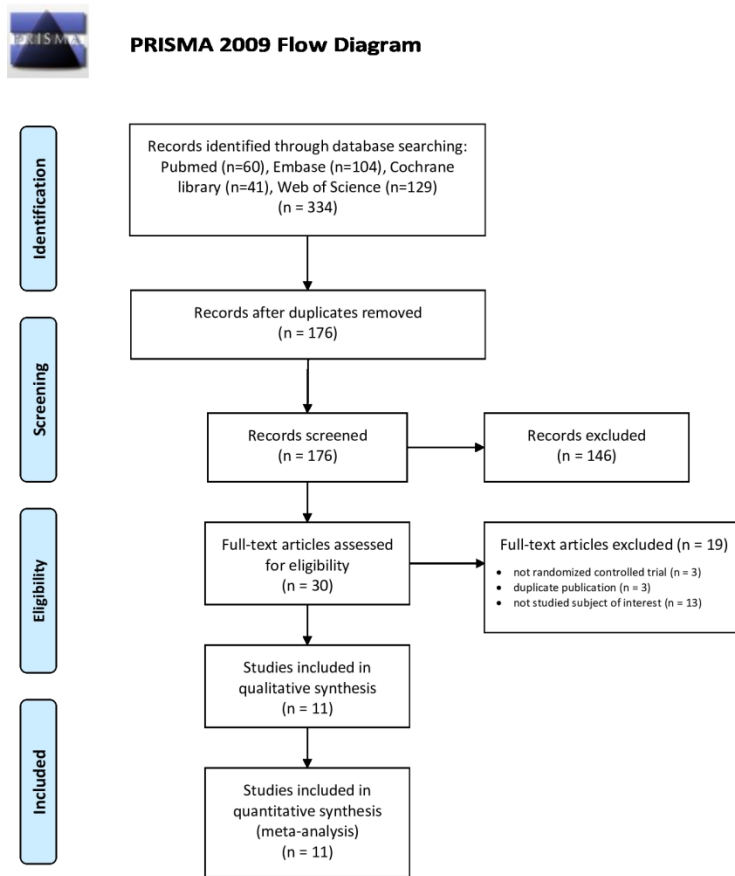


Figure 1: PRISMA flow chart representing the process of the study search and selection

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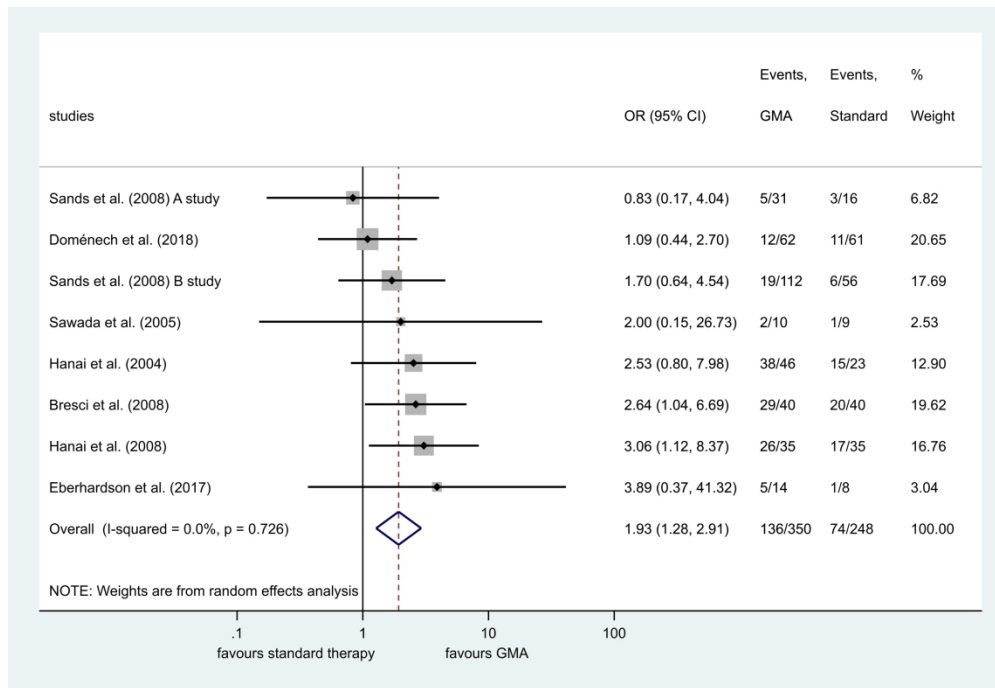


Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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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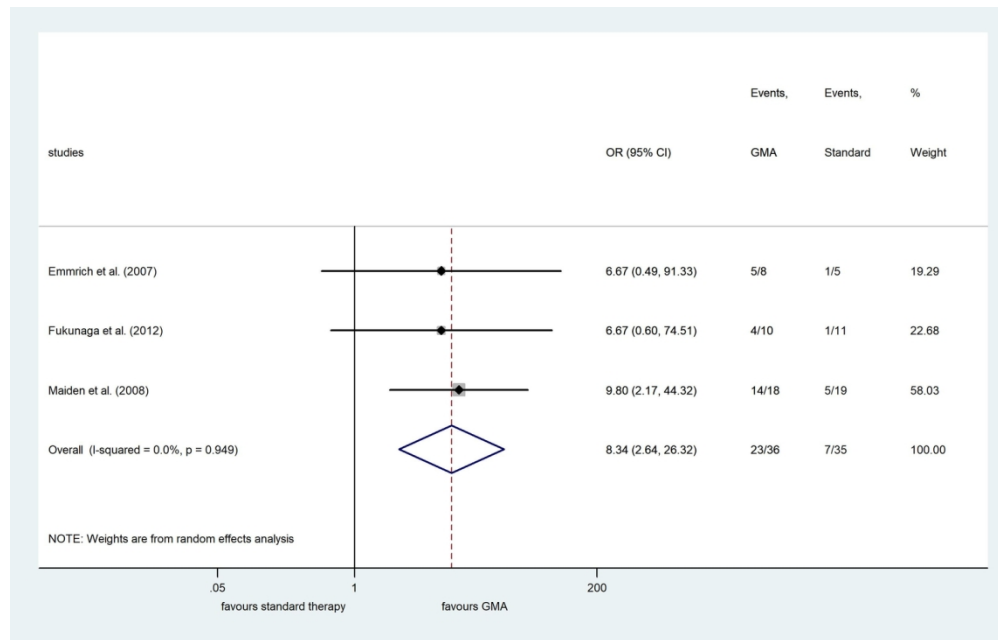


Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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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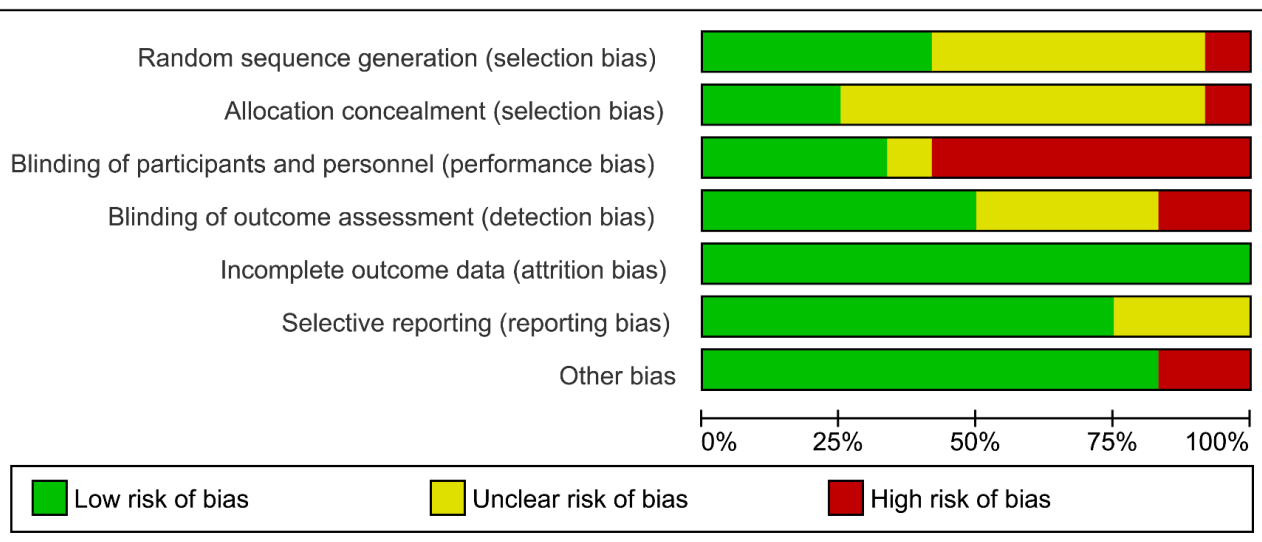
Supplementary Figure 1

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	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Bresci 2008	?	?	-	?	+	+	+
Doménech 2018	+	?	-	-	+	+	+
Eberhardson 2016	?	?	?	+	+	?	+
Emmrich 2006	?	?	-	?	+	?	+
Fukunaga 2012	+	+	+	?	+	+	+
Hanai 2004	?	?	-	+	+	+	+
Hanai 2008	?	?	-	+	+	+	+
Maiden 2012	+	?	-	-	+	?	+
Nakamura 2004	?	?	-	?	+	+	+
Sands 2008 A study	+	+	+	+	+	+	-
Sands 2008 B study	+	+	+	+	+	+	-
Sawada 2005	-	-	+	+	+	+	+

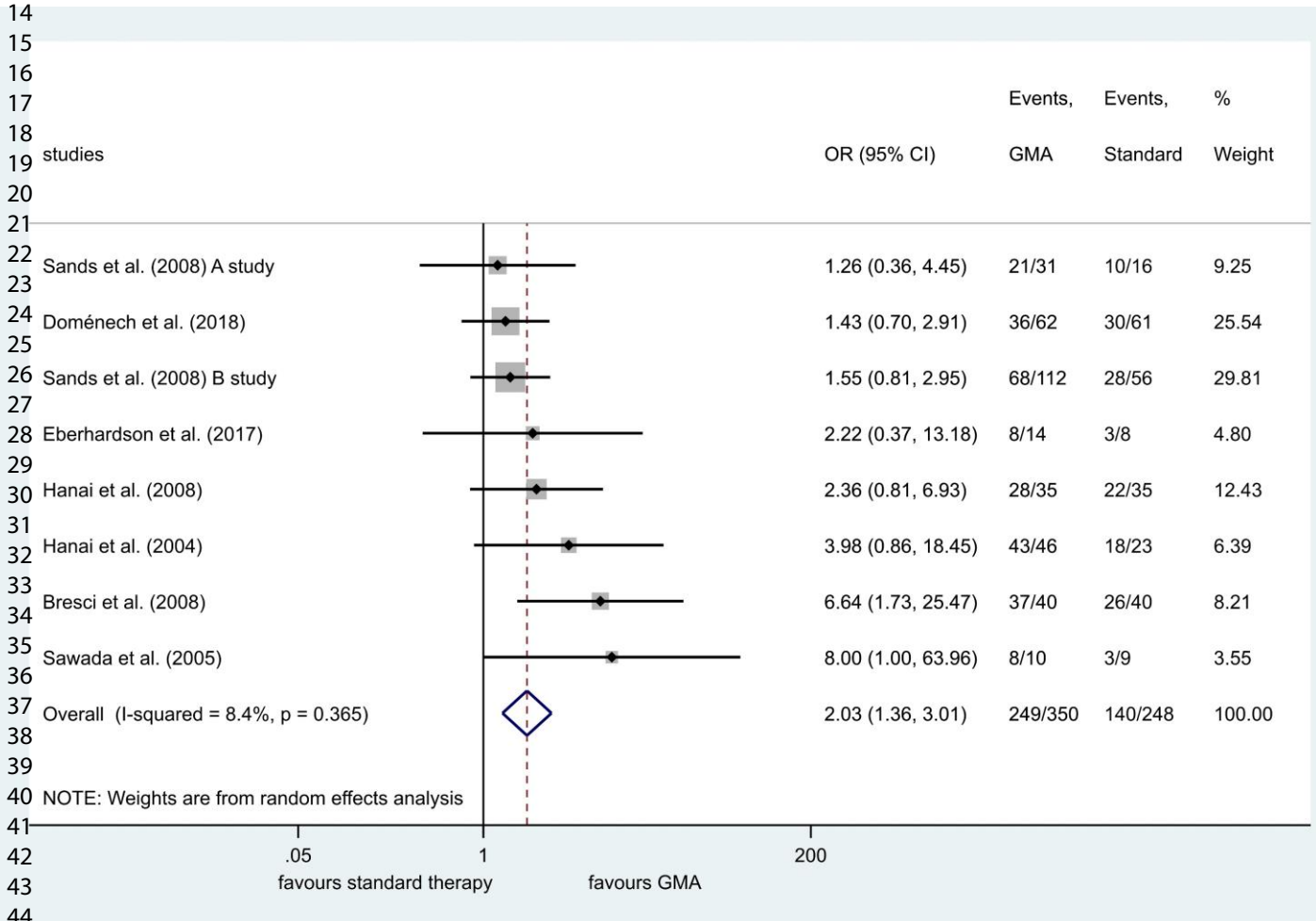
Supplementary Figure 2

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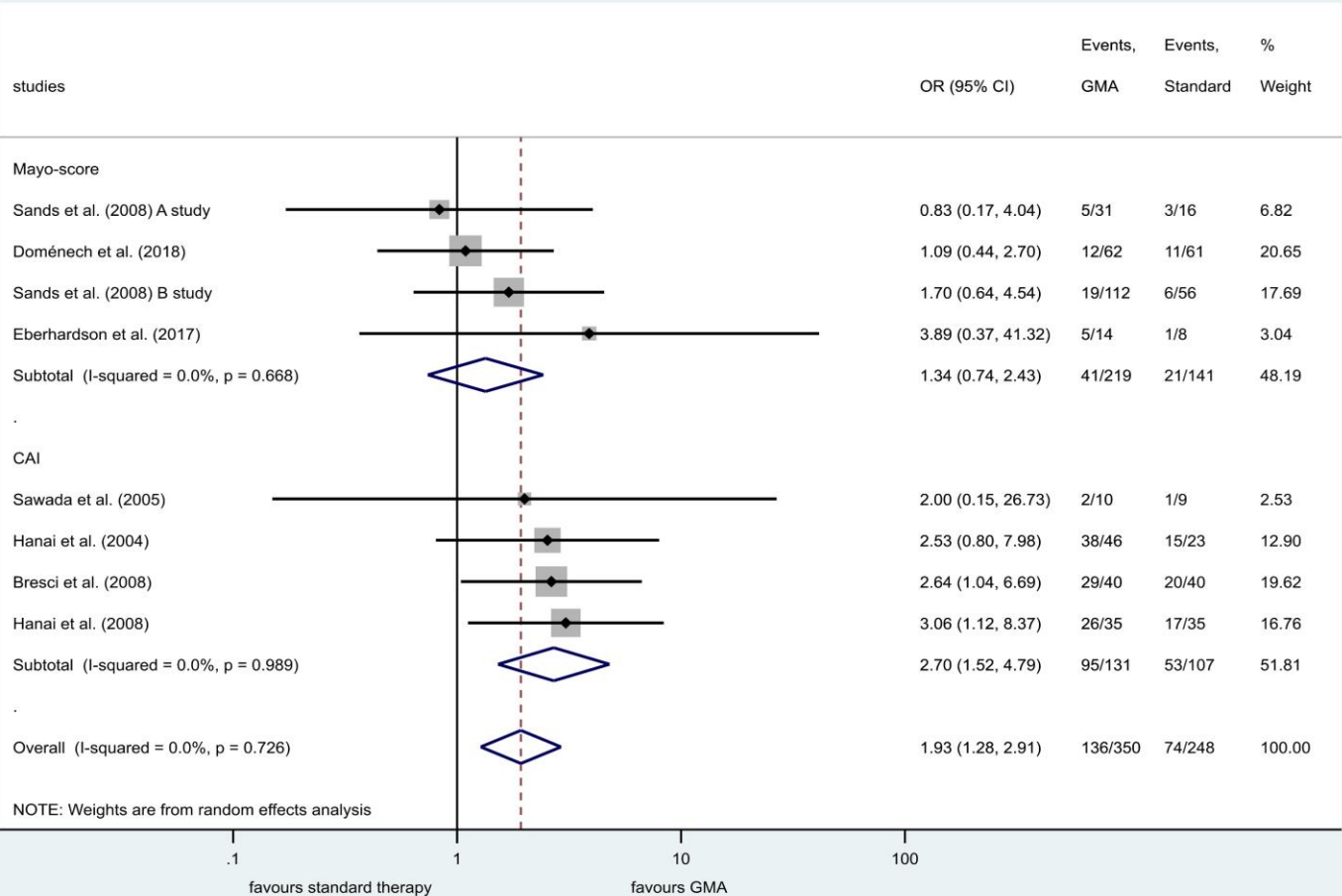
Supplementary Figure 3

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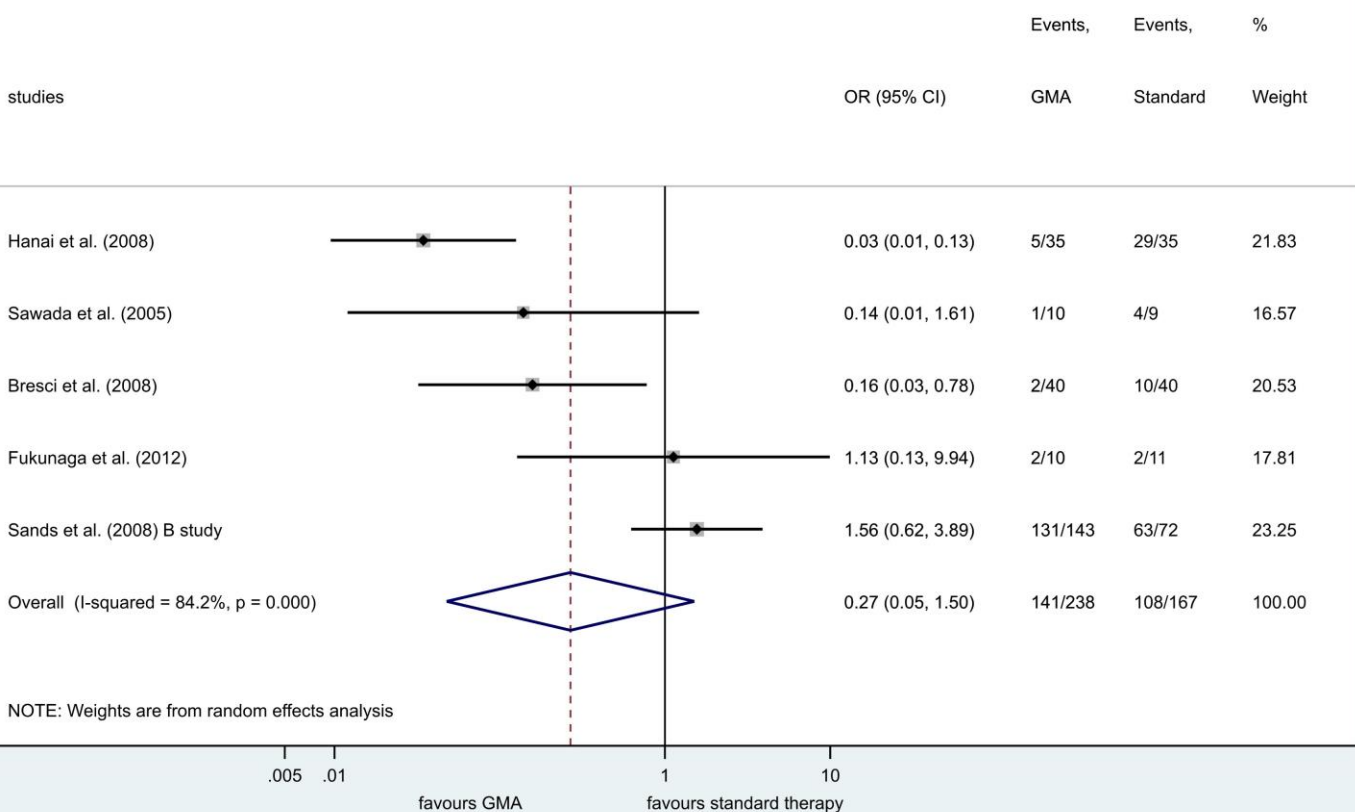
Supplementary Figure 4

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Supplementary Figure 5

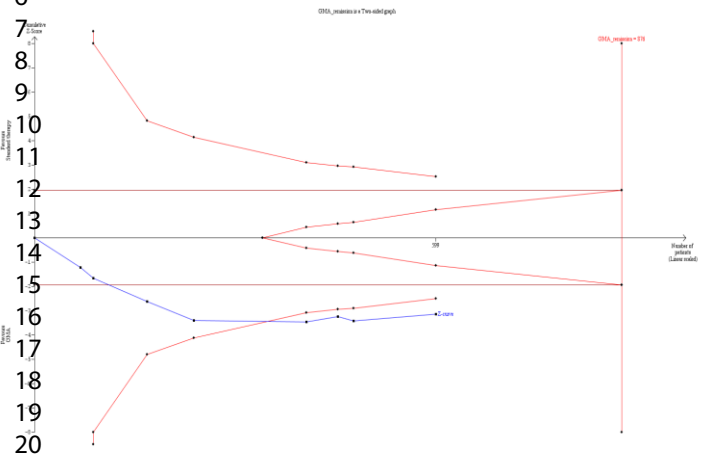
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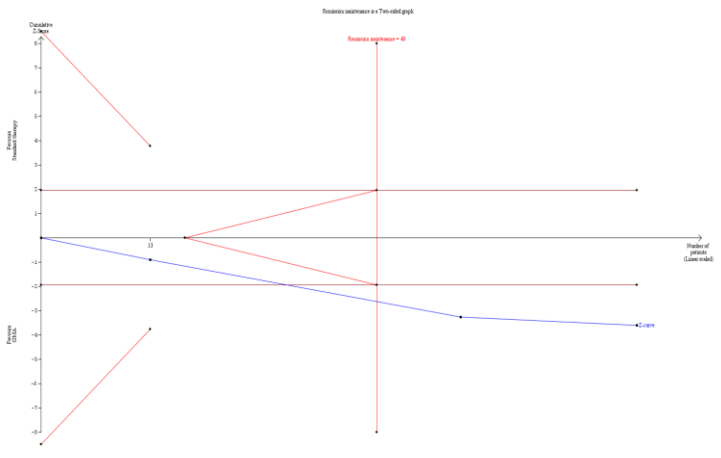
Supplementary Figure 6

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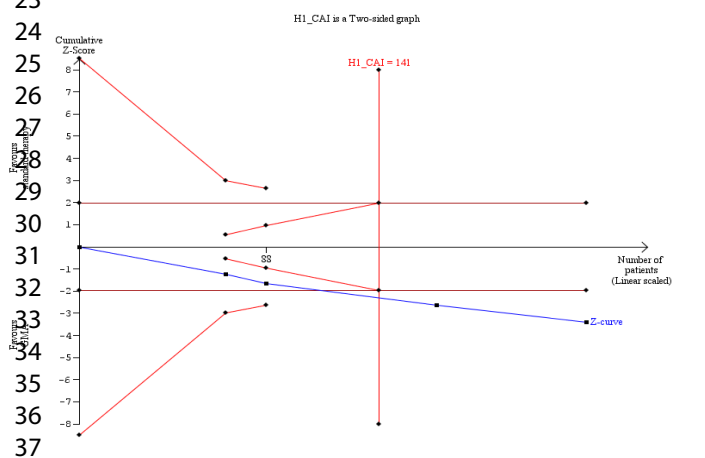
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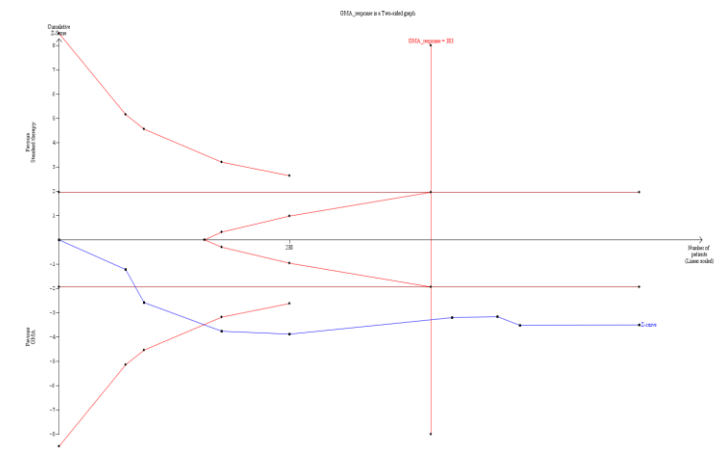
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Supplementary Table 1

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, 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)												
10	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)												
15	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)												
20	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												
25	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												
30	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

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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

For peer review only

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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30
31 15 **Keywords:** Inflammatory bowel disease; IMMUNOLOGY; Gastroenterology;
32 16 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 adjust 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: 4186

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

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

94 4.2 Eligibility criteria

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

98 Specific criteria for clinical remission induction: patients with active UC (*Population₁*),
99 standard therapy for remission induction and GMA as an adjunctive therapy (*Intervention₁*), and
100 standard therapy for remission induction (*Comparison₁*); *Outcomes₁*: clinical response rate and clinical
101 remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.

102 Specific criteria for clinical remission maintenance: patients with UC in clinical remission
103 induced by GMA (*Population₂*), standard therapy for remission maintenance and GMA as an
104 adjunctive therapy (*Intervention₂*), and standard therapy for remission maintenance (*Comparison₂*);
105 *Outcomes₂*: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

106 Outcome criteria for clinical remission and clinical response were defined individually by the
107 eligible articles. These criteria are presented in **Table 1**. Regarding safety, AEs reported by the
108 individual article were used for the analyses in each case. No preliminary specification was made.

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

116 4.3 Data extraction

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

125 4.4 Risk of bias assessment

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

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

- 1
- 2 133 5. Incomplete outcome data (attrition bias)
- 3 134 6. Selective reporting (reporting bias)
- 4 135 7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials,
- 5 136 and imputation of intention-to-treat (ITT) analysis)
- 6

7 137 **4.5 Statistical analysis**

9 138 The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR)
10 139 with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered
11 140 statistically significant (except for heterogeneity, for which a p value <0.10 was considered
12 141 significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR,
13 142 WMD, and weights are presented in forest plots. The random-effects model was used to pool effect
14 143 sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating
15 144 Higgins' I² indicator (13, 14). The Q statistics were computed as the squared deviations from the
16 145 pooled effect of the weighted sum of individual study effects, with the weights being used in the
17 146 pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees
18 147 of freedom (where k was the number of studies). The I² index corresponds to the percentage of the total
19 148 variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane
20 149 Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–
21 150 60%), substantial (50–90%), and considerable (75–100%) (11). Subgroup analysis was performed as
22 151 described in the study protocol if a sufficient number of studies was available. Funnel plots were used
23 152 to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed
24 153 for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal
25 154 information size (OIS). This methodology combines an information size with the threshold of statistical
26 155 significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version
27 156 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).

32 157 **4.6 Quality of evidence**

33 158 The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality
34 159 of evidence for each outcome (15, 16). Disagreements were resolved by consensus.

37 160 **5 Results**

39 161 **5.1 Search and selection**

40 162 The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After
41 163 screening and assessment for eligibility, eleven full-text articles containing one minimized controlled
42 164 trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active
43 165 UC, and three studies contained data on patients with UC in clinical remission.

47 166 **5.2 Characteristics of the studies included**

48 167 The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission
49 168 induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598
50 169 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients
51 170 received GMA, and 248 were in control groups. All the participants had active UC and were treated
52 171 with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received
53 172 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 Cellсорba®. 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,

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2 304 patients achieving clinical remission were allocated to groups with and without GMA treatment for
3 305 remission maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin,
4 306 which is considered as a risk factor of relapse (25). Their results showed that faecal calprotectin level
5 307 significantly decreases following five treatment session. This study differs from the previous two in
6 308 the fact that they enrolled an asymptomatic population regardless of how patients achieved remission.
7 309 The two studies recruiting patients with active UC detected no statistically significant difference
8 310 between study arms in time to first relapse; however, it must be noted that in one of these studies, all
9 311 the patients became steroid-free in the GMA group (9). Maiden et al. found that time to first relapse
10 312 was significantly higher in patients receiving GMA (99 ± 73 days vs. 161 ± 44 days, $p=0.0004$). Despite
11 313 our very promising results, these findings are limited by the amount of available data. More randomized
12 314 controlled trials are necessary in this area to strengthen our results. This study has some potential
13 315 limitations. Allowed concomitant therapies have differed among included studies; therefore, our
14 316 estimates may have been subject to bias, as reflected by the grade of evidence (**Supplementary Table**
15 317 **2**). Moreover, our funnel plots showed symmetry by visual assessment, but publication bias still cannot
16 318 be ruled out because of the low number of included studies. Side-effects and safety data were not
17 319 uniformly reported in most of the publications under analysis, according to the International
18 320 Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines (15). Therefore, our
19 321 second main objective, the safety assessment of GMA, was only achieved to a limited extent.
20 322 Furthermore, this result is strongly limited by the high heterogeneity of studies. The most likely source
21 323 of this is the heterogeneous nature of concomitant treatment. All in all, GMA seems to be a reasonable
22 324 therapeutic option, but finding its exact place to treat UC demands further research. A particularly
23 325 promising area could be remission maintenance.

28 326 **6.1 Conclusion**

30 327 Implications for practice: The results support the hypothesis that patients with active UC have a
31 328 better chance of clinical remission if GMA is administered as an adjunctive therapy. As regards the
32 329 frequency of AEs, we found no statistically significant difference between the two groups. With
33 330 regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

34 331 Implications for research: Further studies are required to select patients who may benefit the most
35 332 from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its
36 333 role in remission induction. There is currently evidence available about induction and maintenance of
37 334 clinical remission; however, the role of GMA concerning endoscopic and histological remission is
38 335 currently unclear. If GMA is proven to be safe and effective, cost-effectiveness studies will also be
39 336 worthwhile in the future.

44 337 **7 Data availability statement**

45 338 The data that support the findings of this study are available from the corresponding author, [A.H.],
46 339 upon reasonable request.

49 340 **8 Patient and Public Involvement**

50 341 It was not appropriate or possible to involve patients or the public in the design, or conduct, or
51 342 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.

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48 499 15 Figures and tables

49
50 500 **Figure 1:** PRISMA flow chart representing the process of the study search and selection

51
52 501 **Figure 2:** Forest plot of studies comparing clinical remission induction between patients with and
53 502 without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and
54 503 vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on
55 504 the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of
56 505 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

541 **Table 1: Characteristics of included studies**

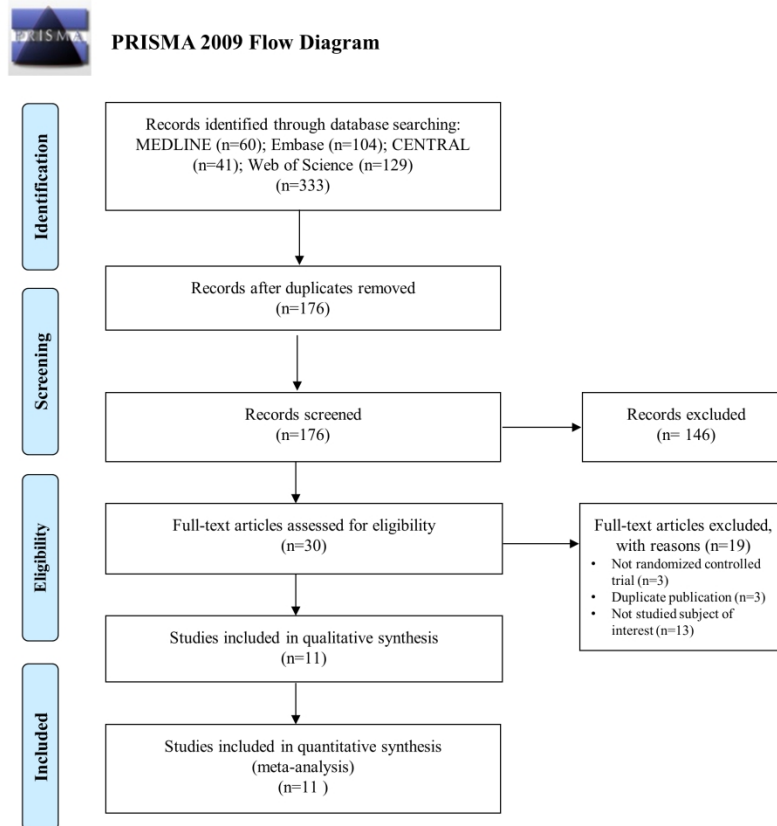
542 * All patients received standard of care added to investigator/comparator. 1: one patient was excluded from analysis because of
 543 protocol deviations; 2: one patient was excluded from analysis because of protocol deviations; 3: one patient was excluded due to
 544 failure to return blood from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

Clinical remission induction											
Study Name and Setting	N ^o of cycles (n)	Randomization*	N ^o of patients analyzed (n)	Patients achieving response		Patients achieving remission		Time of assessment	Outcome criteria		Concomitant medication
				%	n	%	n		Remission	Response	
Brescia 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 single 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			



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

For more information, visit www.prisma-statement.org.

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

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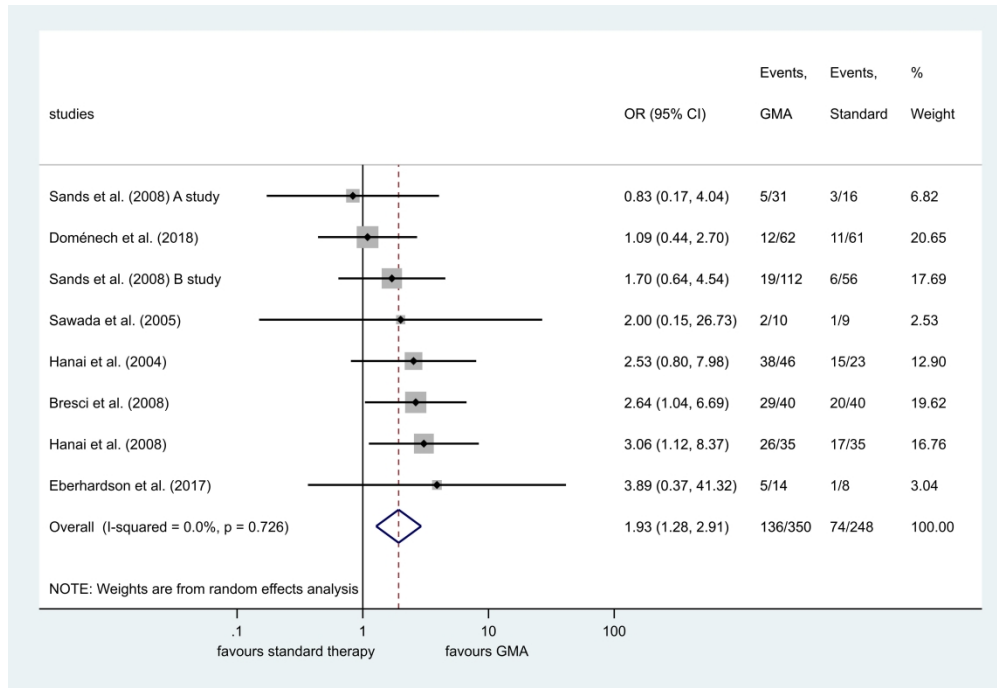


Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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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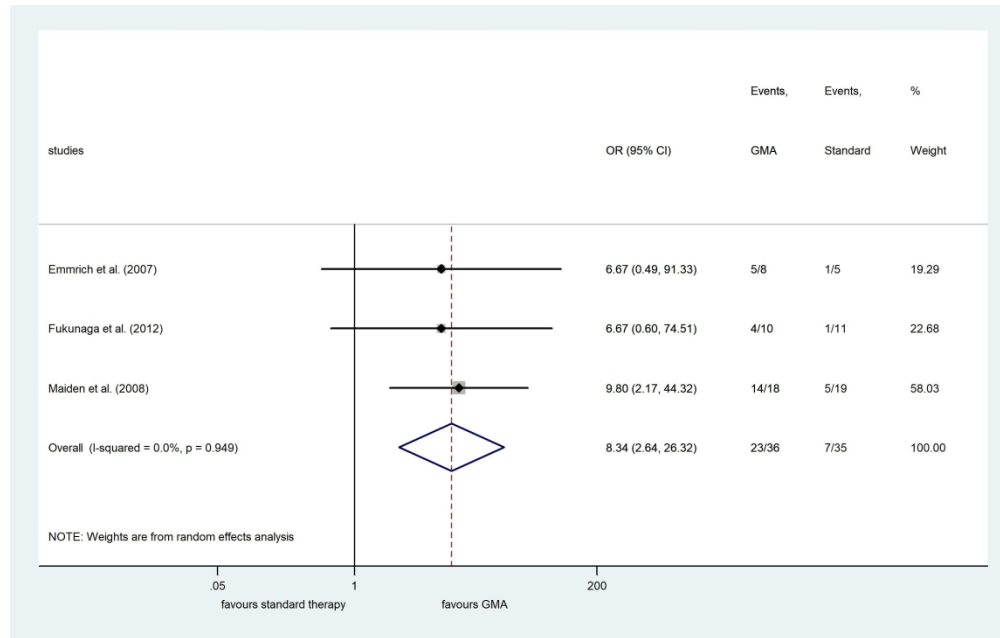


Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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 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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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.

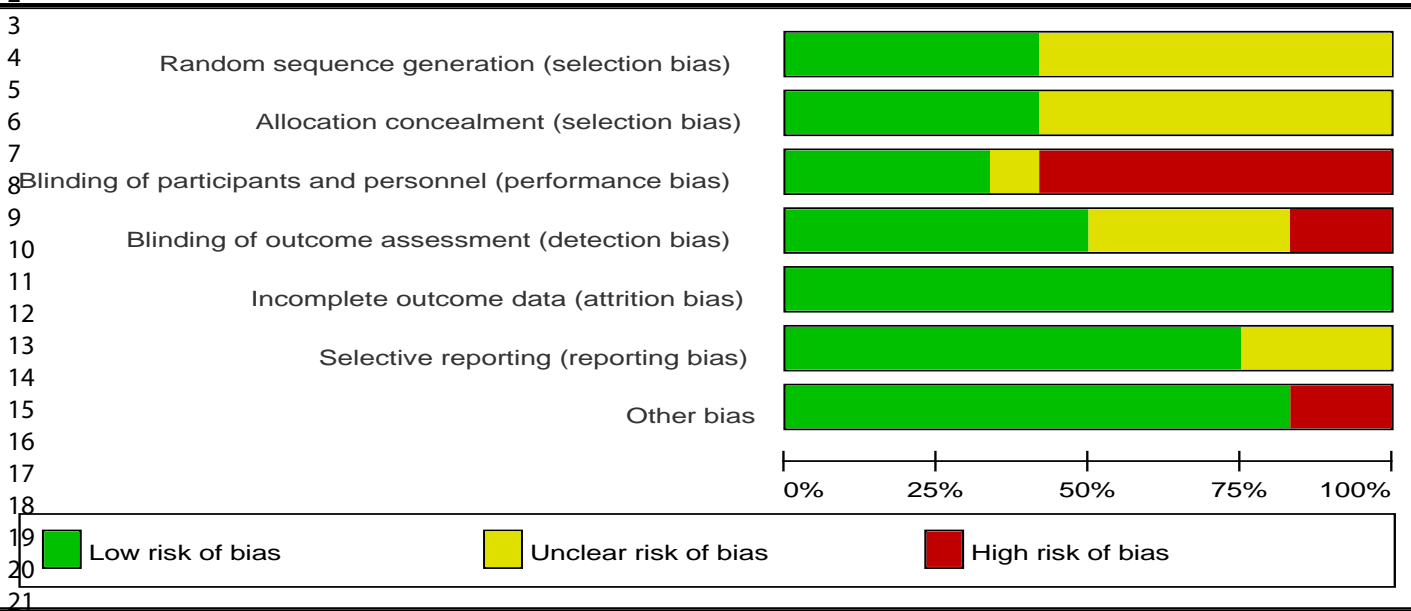
Search	Query	Automatic explosion
#1	gma OR apheresis OR adsorption OR "cell separation" OR leukapher* OR leukopher* OR leukocytapher* OR leukocytopher* OR lymphapher* OR lymphopher* OR lymphocytopher* OR lymphocytapher*	("gma"[All Fields] OR ("blood component removal"[MeSH Terms] OR ("blood"[All Fields] 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]

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	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Bresci 2008	?	?	-	?	+	+	+
Doménech 2018	+	+	-	-	+	+	+
Eberhardson 2016	?	?	?	+	+	?	+
Emmrich 2006	?	?	-	?	+	?	+
Fukunaga 2012	+	+	+	?	+	+	+
Hanai 2004	?	?	-	+	+	+	+
Hanai 2008	?	?	-	+	+	+	+
Maiden 2012	+	?	-	-	+	?	+
Nakamura 2004	?	?	-	?	+	+	+
Sands 2008 A study	+	+	+	+	+	+	-
Sands 2008 B study	+	+	+	+	+	+	-
Sawada 2005	?	+	+	+	+	+	+

Supplementary Figure 2

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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: "...randomization codes were centrally generated using a computer procedure..." Blocked randomization was used.
Allocation concealment (selection bias)	Low risk	Quote: "...randomization codes were centrally 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.

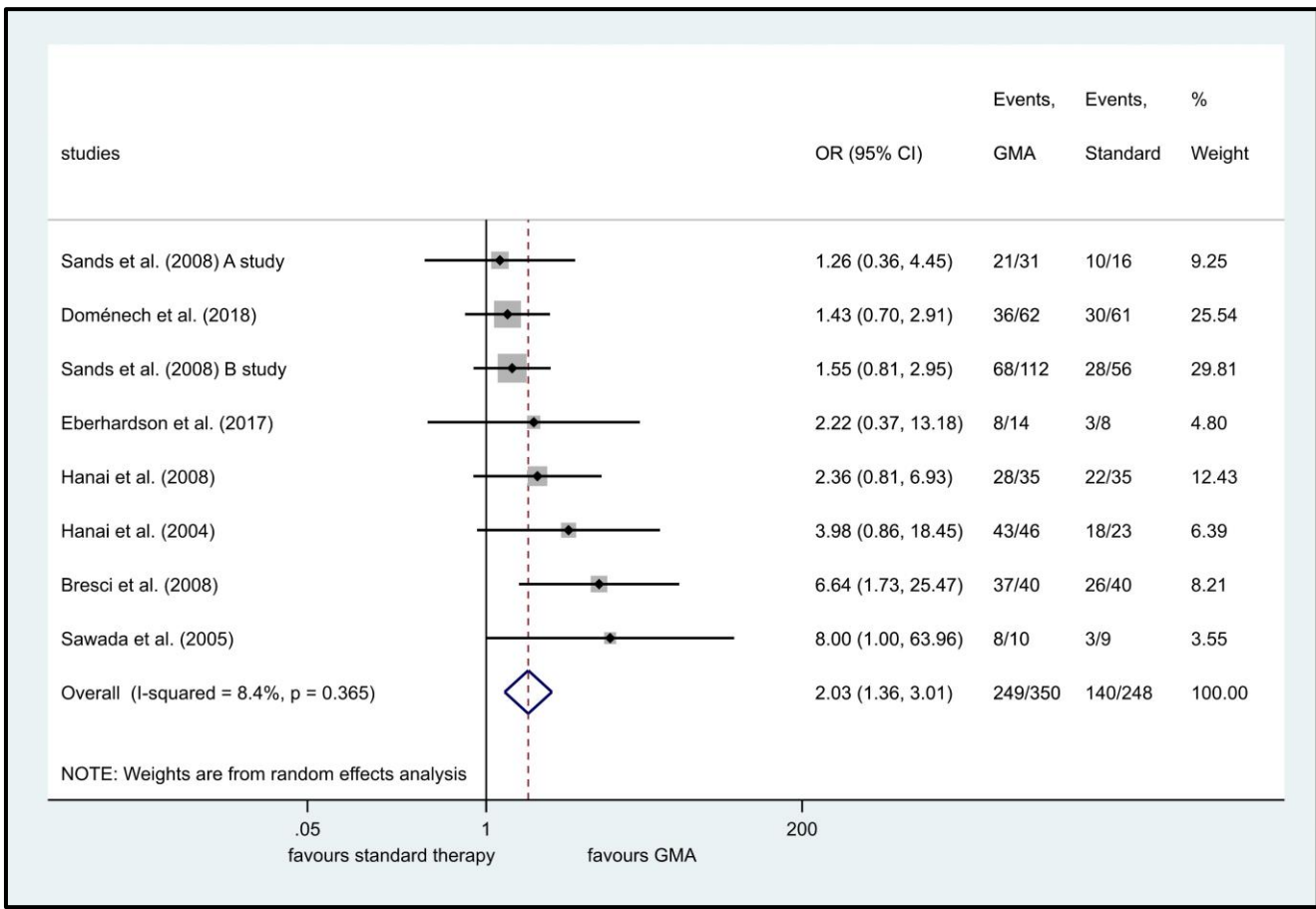
Fukunaga et al. 2012	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Blocked randomization according to a computer-generated scheme.
Allocation concealment (selection bias)	Low risk	Patients were randomized in a 1:1:1 ratio by a statistician at an independent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

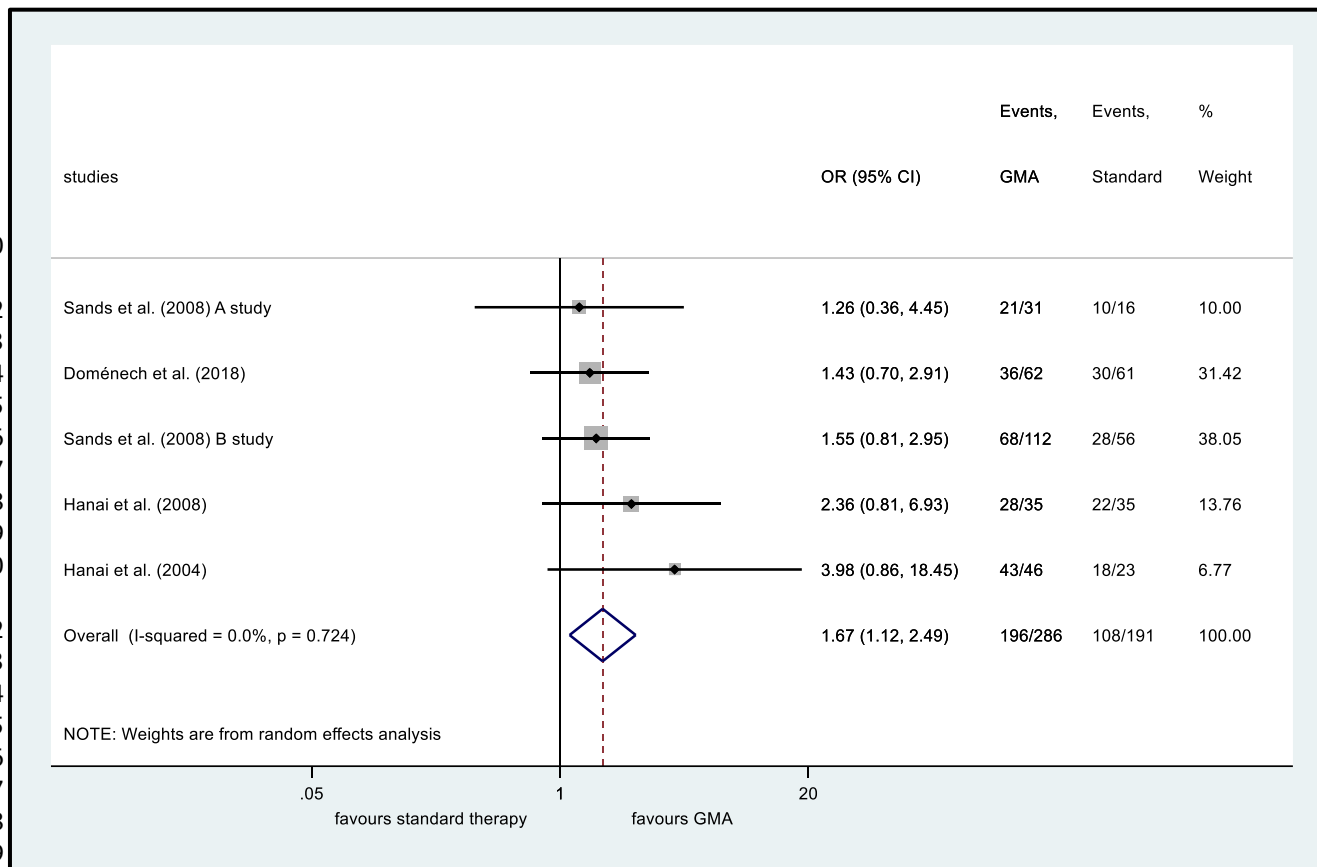
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	21/22 completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Unclear 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

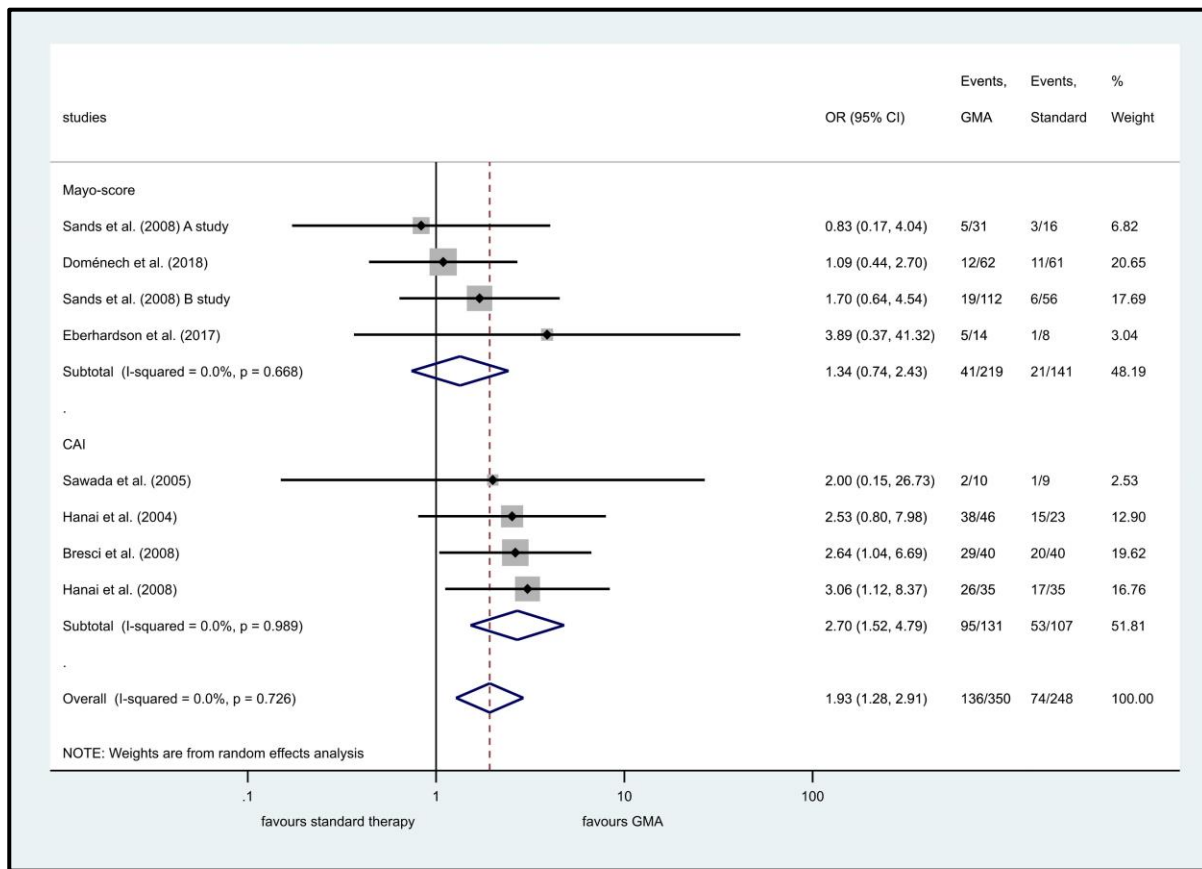
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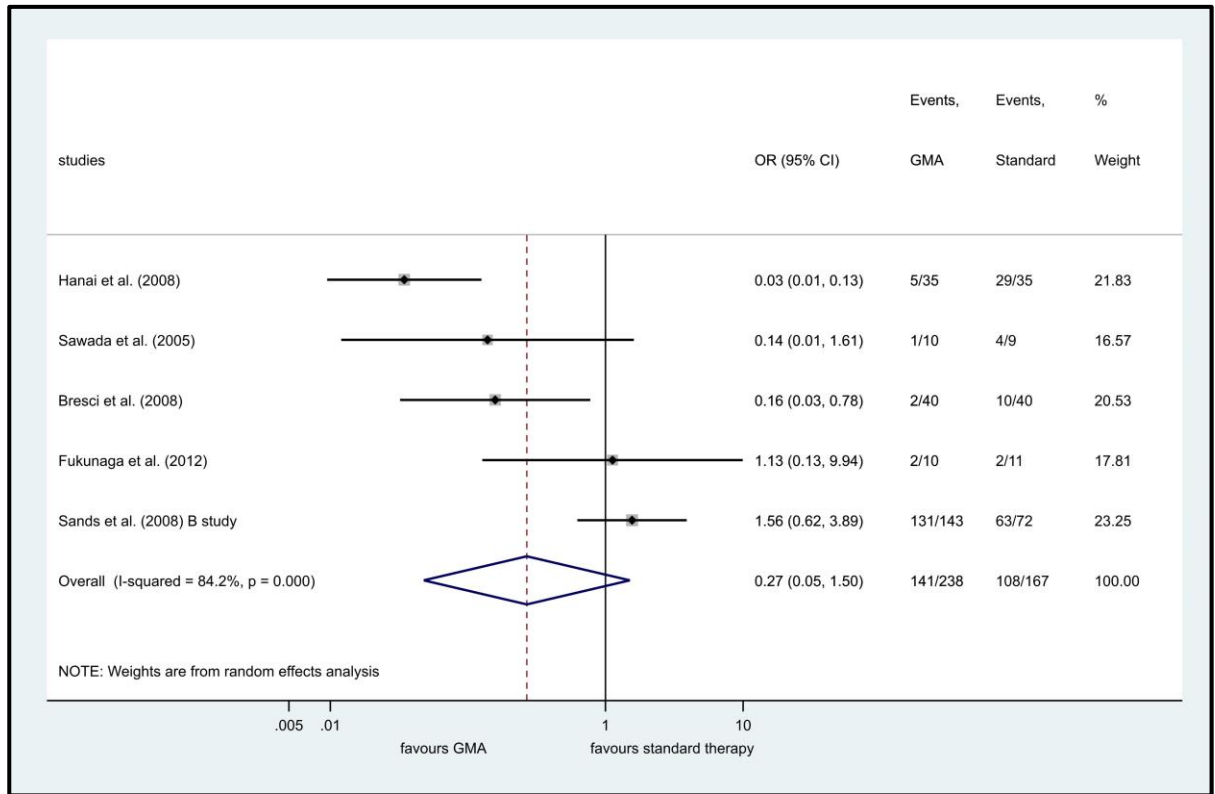




Supplementary Figure 5

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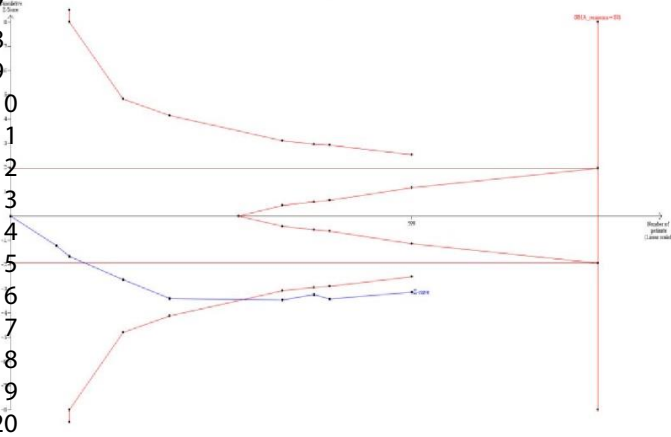


Supplementary Figure 7

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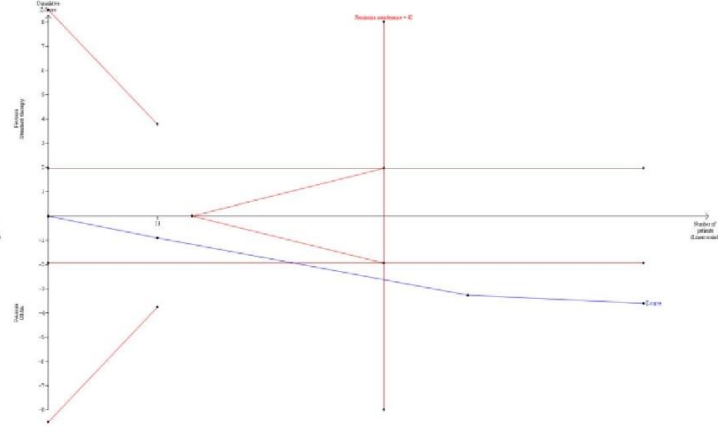
A

SDI_patient is a Two-sided graph



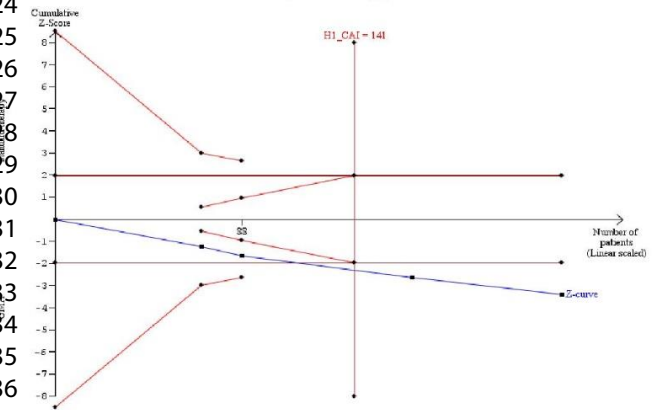
B

Residence satisfaction is a Two-sided graph



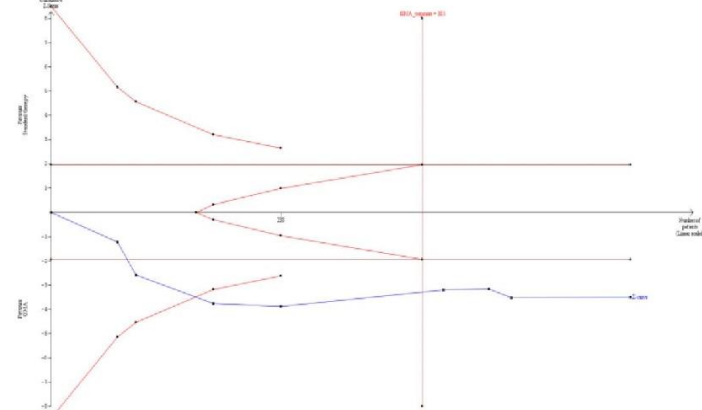
C

H1_CAI is a Two-sided graph



D

SDI_patient is a Two-sided graph



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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Page 2 of 2

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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30
31 15 **Keywords:** Inflammatory bowel disease; IMMUNOLOGY; Gastroenterology;
32 16 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 adjust 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

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

94 4.2 Eligibility criteria

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

98 Specific criteria for clinical remission induction: patients with active UC (*Population₁*),
99 standard therapy for remission induction and GMA as an adjunctive therapy (*Intervention₁*), and
100 standard therapy for remission induction (*Comparison₁*); *Outcomes₁*: clinical response rate and clinical
101 remission rate (defined either by the clinical activity index (CAI) or full Mayo score) and AEs.

102 Specific criteria for clinical remission maintenance: patients with UC in clinical remission
103 induced by GMA (*Population₂*), standard therapy for remission maintenance and GMA as an
104 adjunctive therapy (*Intervention₂*), and standard therapy for remission maintenance (*Comparison₂*);
105 *Outcomes₂*: rate of maintained remission (defined either by the CAI or full Mayo score) and AEs.

106 Outcome criteria for clinical remission and clinical response were defined individually by the
107 eligible articles. These criteria are presented in **Table 1**. Regarding safety, AEs reported by the
108 individual article were used for the analyses in each case. No preliminary specification was made.

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

116 4.3 Data extraction

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

125 4.4 Risk of bias assessment

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

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

- 1
- 2 133 5. Incomplete outcome data (attrition bias)
- 3 134 6. Selective reporting (reporting bias)
- 4 135 7. Other bias (early stopping, baseline imbalance, blocked randomization with unblinded trials,
- 5 136 and imputation of intention-to-treat (ITT) analysis)
- 6

7 137 **4.5 Statistical analysis**

9 138 The effect measure of dichotomous variables was reported for each outcome as the odds ratio (OR)
10 139 with the related 95% confidence interval (8). All tests were 2-sided, and a p value <0.05 was considered
11 140 statistically significant (except for heterogeneity, for which a p value <0.10 was considered
12 141 significant). Weighted mean difference (WMD) was calculated for continuous variables. Values of OR,
13 142 WMD, and weights are presented in forest plots. The random-effects model was used to pool effect
14 143 sizes. Heterogeneity was tested both by performing Cochran's Q test and calculating
15 144 Higgins' I² indicator (13, 14). The Q statistics were computed as the squared deviations from the
16 145 pooled effect of the weighted sum of individual study effects, with the weights being used in the
17 146 pooling method. P values were obtained by comparing test statistics with a chi-square with k-1 degrees
18 147 of freedom (where k was the number of studies). The I² index corresponds to the percentage of the total
19 148 variability across studies due to heterogeneity. A rough classification of its value based on the Cochrane
20 149 Handbook for Systematic Reviews of Interventions is the following: low (0–40%), moderate (30–
21 150 60%), substantial (50–90%), and considerable (75–100%) (11). Subgroup analysis was performed as
22 151 described in the study protocol if a sufficient number of studies was available. Funnel plots were used
23 152 to test the presence of publication bias. A Trial Sequential Analysis (TSA 0.9.5.10.) was also performed
24 153 for the randomized controlled studies to quantify the statistical reliability and to estimate the optimal
25 154 information size (OIS). This methodology combines an information size with the threshold of statistical
26 155 significance. All the statistical analyses were performed using Comprehensive Meta-Analysis (version
27 156 3, Biostat Inc., Englewood, NJ, USA) and StataIC (version 15.1).

32 157 **4.6 Quality of evidence**

33 158 The GRADE approach was used by the two independent reviewers (KS and FM) to assess the quality
34 159 of evidence for each outcome (15, 16). Disagreements were resolved by consensus.

37 160 **5 Results**

39 161 **5.1 Search and selection**

40 162 The search process is shown in **Figure 1**. A total of 334 records were identified in the databases. After
41 163 screening and assessment for eligibility, eleven full-text articles containing one minimized controlled
42 164 trial and eleven RCTs were included for analysis. Eight studies provided data on patients with active
43 165 UC, and three studies contained data on patients with UC in clinical remission.

47 166 **5.2 Characteristics of the studies included**

48 167 The characteristics of the included studies are presented in **Table 1**. In the case of clinical remission
49 168 induction, all the studies were RCTs, except for the one study with minimization (17). A total of 598
50 169 participants (mean: 77, ranging from 19 to 168) were included in this meta-analysis: 350 patients
51 170 received GMA, and 248 were in control groups. All the participants had active UC and were treated
52 171 with Adacolumn® (7, 17-23). Four of these trials were sham-controlled. All the patients received
53 172 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 Cellсорba®. 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

1
2 304 UC patients based on CAI (9, 24). After successful induction therapy with the inclusion of GMA,
3 305 patients achieving clinical remission were allocated to groups with and without GMA treatment for
4 306 remission maintenance. Maiden et al. enrolled UC patients with a high level of faecal calprotectin,
5 307 which is considered as a risk factor of relapse (25). Their results showed that faecal calprotectin level
6 308 significantly decreases following five treatment session. This study differs from the previous two in
7 309 the fact that they enrolled an asymptomatic population regardless of how patients achieved remission.
8 310 The two studies recruiting patients with active UC detected no statistically significant difference
9 311 between study arms in time to first relapse; however, it must be noted that in one of these studies, all
10 312 the patients became steroid-free in the GMA group (9). Maiden et al. found that time to first relapse
11 313 was significantly higher in patients receiving GMA (99 ± 73 days vs. 161 ± 44 days, $p=0.0004$). Despite
12 314 our very promising results, these findings are limited by the amount of available data. More randomized
13 315 controlled trials are necessary in this area to strengthen our results. This study has some potential
14 316 limitations. Allowed concomitant therapies have differed among included studies; therefore, our
15 317 estimates may have been subject to bias, as reflected by the grade of evidence (**Supplementary Table**
16 318 **2**). Moreover, our funnel plots showed symmetry by visual assessment, but publication bias still cannot
17 319 be ruled out because of the low number of included studies. Side-effects and safety data were not
18 320 uniformly reported in most of the publications under analysis, according to the International
19 321 Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines (15). Therefore, our
20 322 second main objective, the safety assessment of GMA, was only achieved to a limited extent.
21 323 Furthermore, this result is strongly limited by the high heterogeneity of studies. The most likely source
22 324 of this is the heterogeneous nature of concomitant treatment. All in all, GMA seems to be a reasonable
23 325 therapeutic option, but finding its exact place to treat UC demands further research. A particularly
24 326 promising area could be remission maintenance.

29 327 **6.1 Conclusion**

31 328 Implications for practice: The results support the hypothesis that patients with active UC have a
32 329 better chance of clinical remission if GMA is administered as an adjunctive therapy. As regards the
33 330 frequency of AEs, we found no statistically significant difference between the two groups. With
34 331 regard to remission maintenance, GMA was identified as an effective alternative therapeutic option.

35 332 Implications for research: Further studies are required to select patients who may benefit the most
36 333 from GMA therapy. Nevertheless, more randomized controlled studies are necessary to justify its
37 334 role in remission induction. There is currently evidence available about induction and maintenance of
38 335 clinical remission; however, the role of GMA concerning endoscopic and histological remission is
39 336 currently unclear. If GMA is proven to be safe and effective, cost-effectiveness studies will also be
40 337 worthwhile in the future.

45 338 **7 Data availability statement**

46
47 339 The data that support the findings of this study are available from the corresponding author, [A.H.],
48 340 upon reasonable request.

50 341 **8 Patient and Public Involvement**

51
52 342 It was not appropriate or possible to involve patients or the public in the design, or conduct, or
53 343 reporting, or dissemination plans of our research.

344 9 Author contributions

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

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367 11 Conflict of interest

368 Authors do not have any conflicts of interest to declare.

369 12 Compliance with Ethical Standards

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

373 13 Abbreviations

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

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48 500 15 Figures and tables

49
50 501 **Figure 1:** PRISMA flow chart representing the process of the study search and selection

51
52 502 **Figure 2:** Forest plot of studies comparing clinical remission induction between patients with and
53 503 without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and
54 504 vertical lines show the corresponding 95% confidence intervals. Size of the grey squares reflect on
55 505 the weight of a particular study. The blue diamond the overall or summary effect. The outer edges of
56 506 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. 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

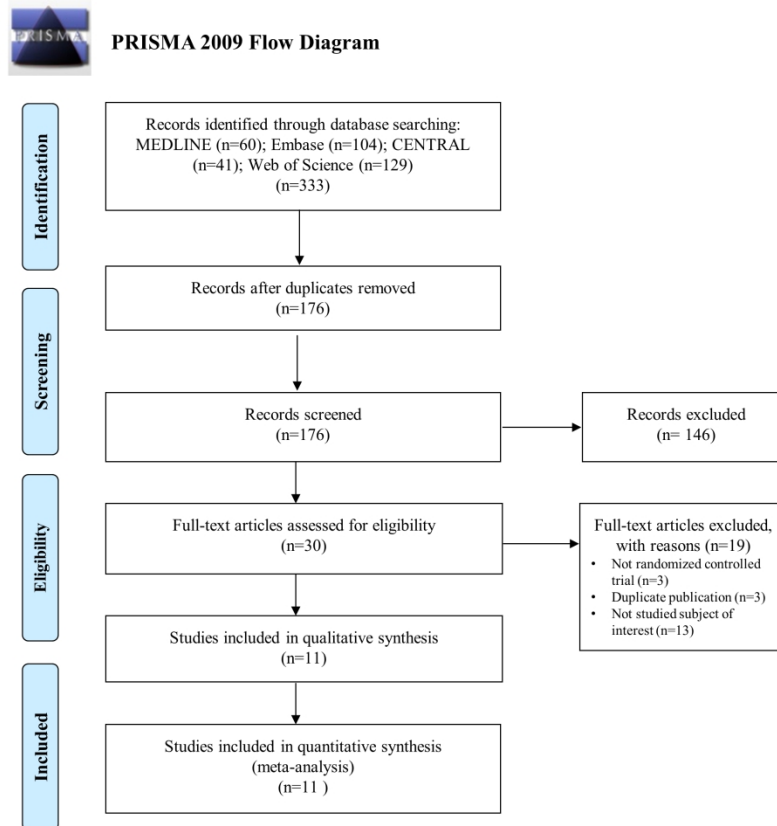
* All patients received standard of care added to investigator/comparator. 1: one patient was excluded from analysis because of protocol deviations; 2: one patient was excluded from analysis because of protocol deviations; 3: one patient was excluded due to failure to return blood from the column; 4: 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	N ^o of cycles (n)	Randomization*	N ^o 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 single 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			



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

For more information, visit www.prisma-statement.org.

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

190x275mm (300 x 300 DPI)

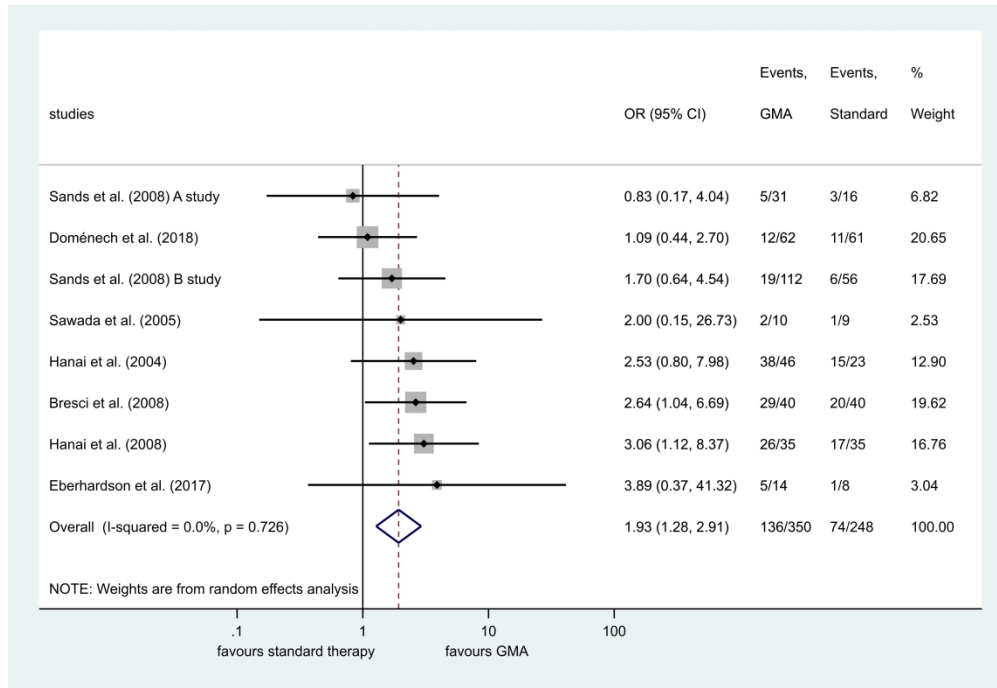


Figure 2: Forest plot of studies comparing clinical remission induction between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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)

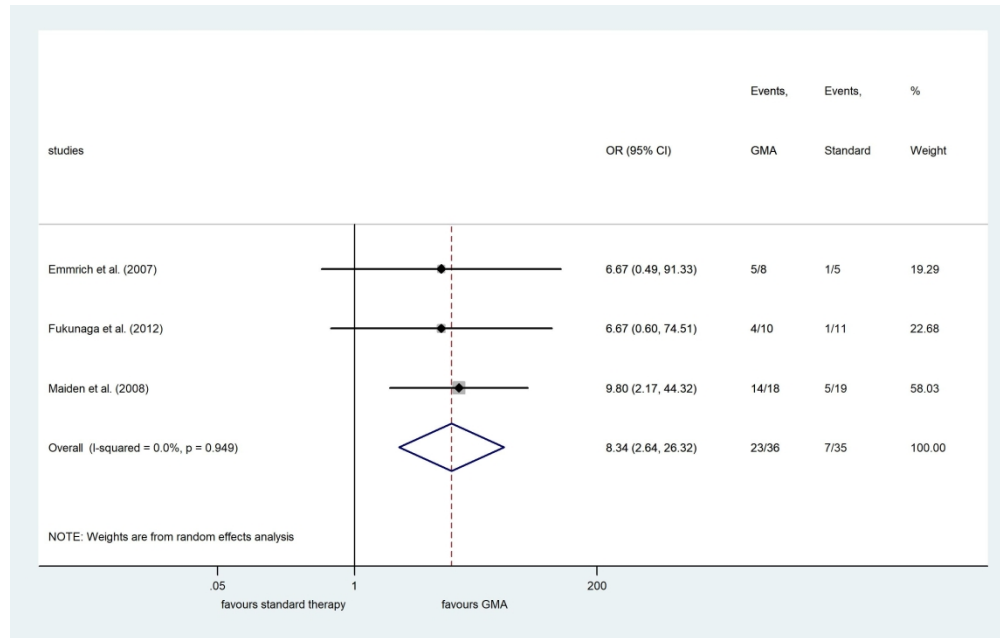


Figure 3: Forest plot of studies comparing clinical remission maintenance between patients with and without GMA as adjunctive therapy. Black diamonds represent the individual studies effect and vertical lines show the corresponding 95% confidence intervals (CI). Size of the grey squares reflect on the 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)

Supplementary 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 Németh², Péter Hegyi^{2,3}, Mária Földi^{1,2}, Zsolt Szakács^{2,3}, Bálint Erőss^{2,3}, Benedek Tinusz⁴, 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*)

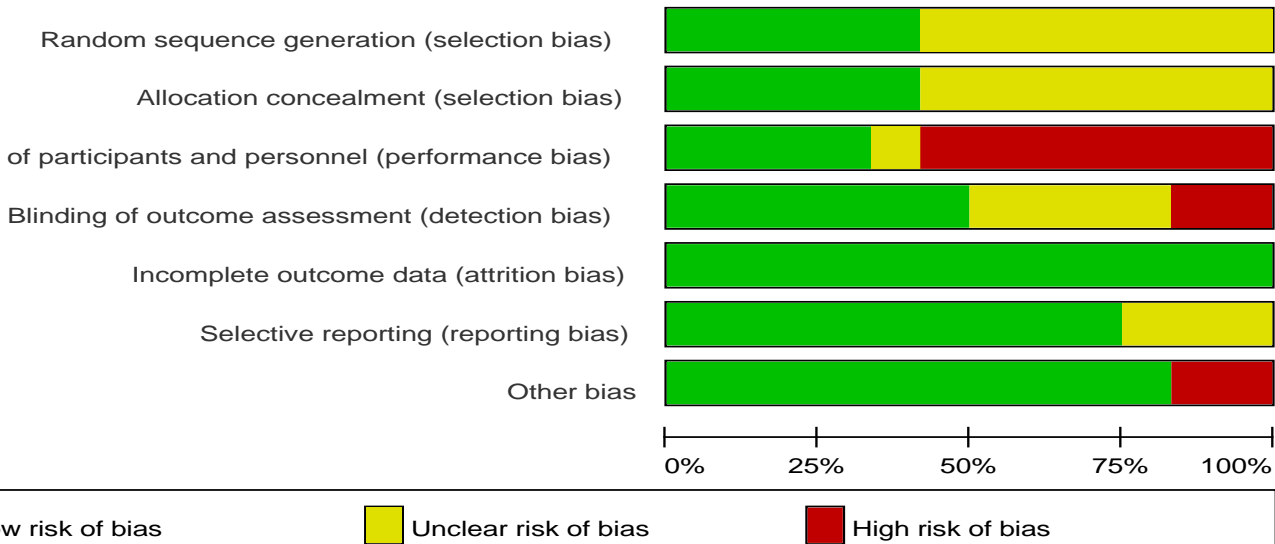
No filters or restrictions were applied.

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]

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Bresci 2008	?	?	-	?	+	+	+
Doménech 2018	+	+	-	-	+	+	+
Eberhardson 2016	?	?	?	+	+	?	+
Emmrich 2006	?	?	-	?	+	?	+
Fukunaga 2012	+	+	+	?	+	+	+
Hanai 2004	?	?	-	+	+	+	+
Hanai 2008	?	?	-	+	+	+	+
Maiden 2012	+	?	-	-	+	?	+
Nakamura 2004	?	?	-	?	+	+	+
Sands 2008 A study	+	+	+	+	+	+	-
Sands 2008 B study	+	+	+	+	+	+	-
Sawada 2005	?	+	+	+	+	+	+

Supplementary Figure 2

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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: "...randomization codes were centrally generated using a computer procedure..." Blocked randomization was used.
Allocation concealment (selection bias)	Low risk	Quote: "...randomization codes were centrally 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.

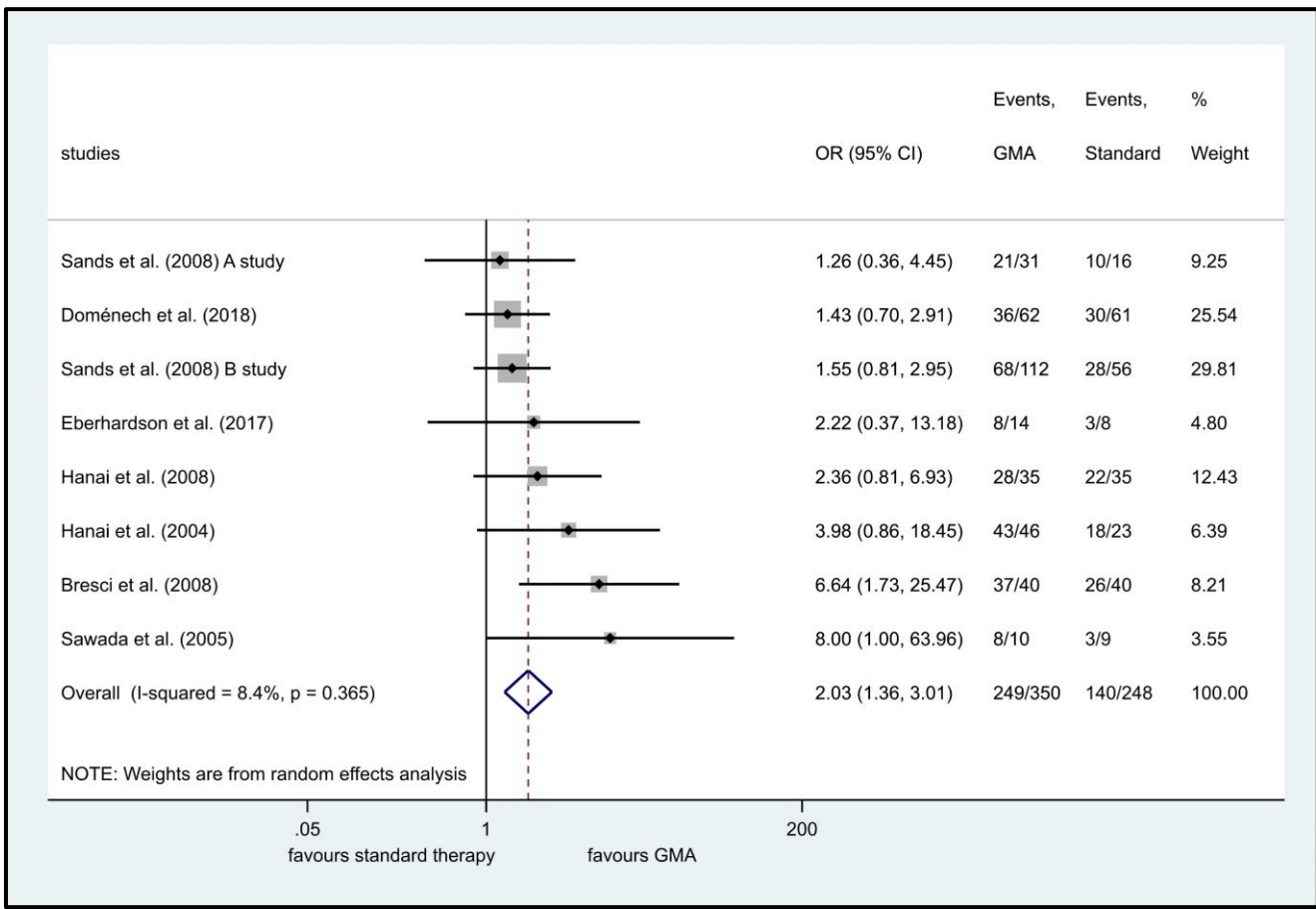
Fukunaga et al. 2012	Authors judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Blocked randomization according to a computer-generated scheme.
Allocation concealment (selection bias)	Low risk	Patients were randomized in a 1:1:1 ratio by a statistician at an independent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

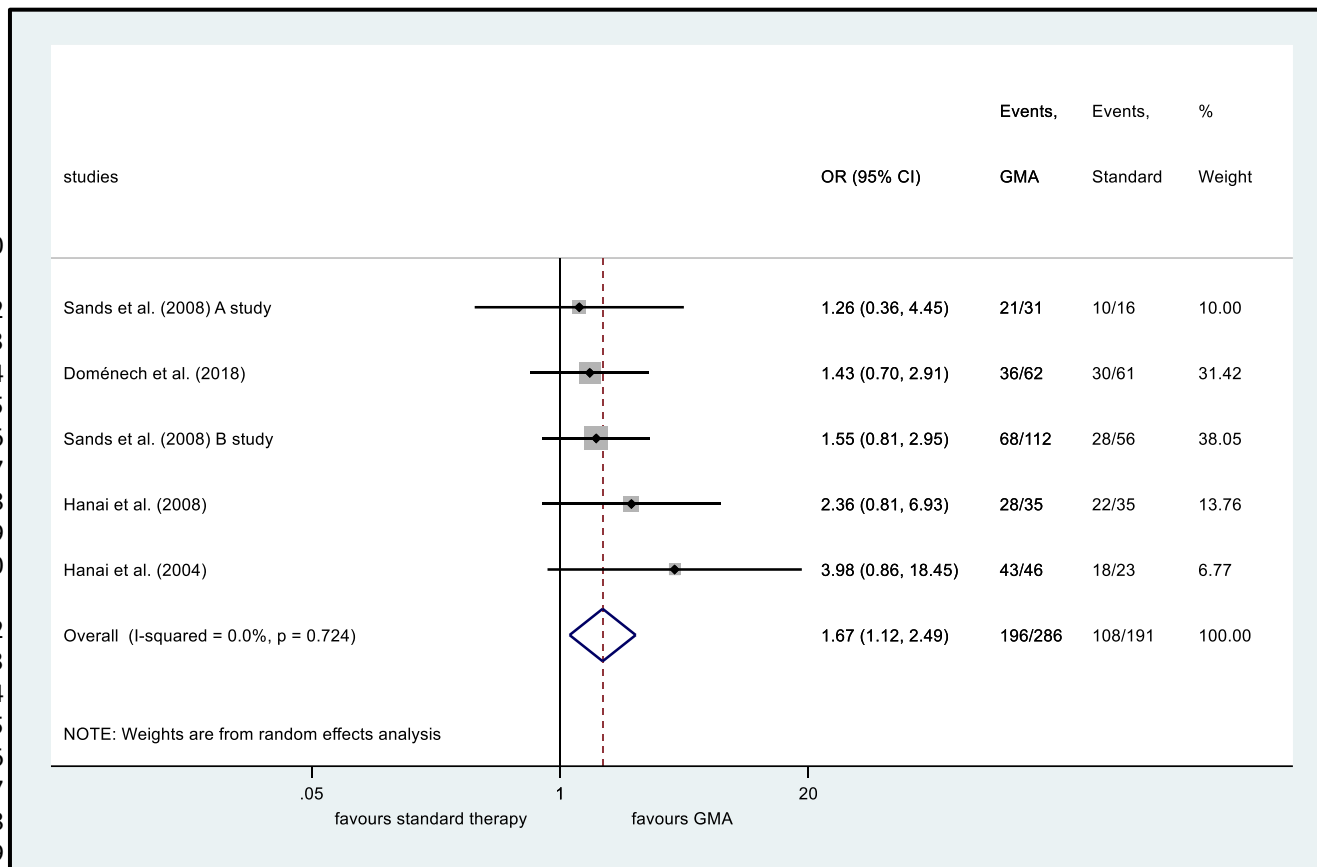
Blinding of outcome assessment (detection bias)	Unclear risk	Not described in the manuscript.
Incomplete outcome data (attrition bias)	Low risk	21/22 completed the study.
Selective reporting (reporting bias)	Low risk	Both significant and non-significant results have been reported.
Other bias	Unclear 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

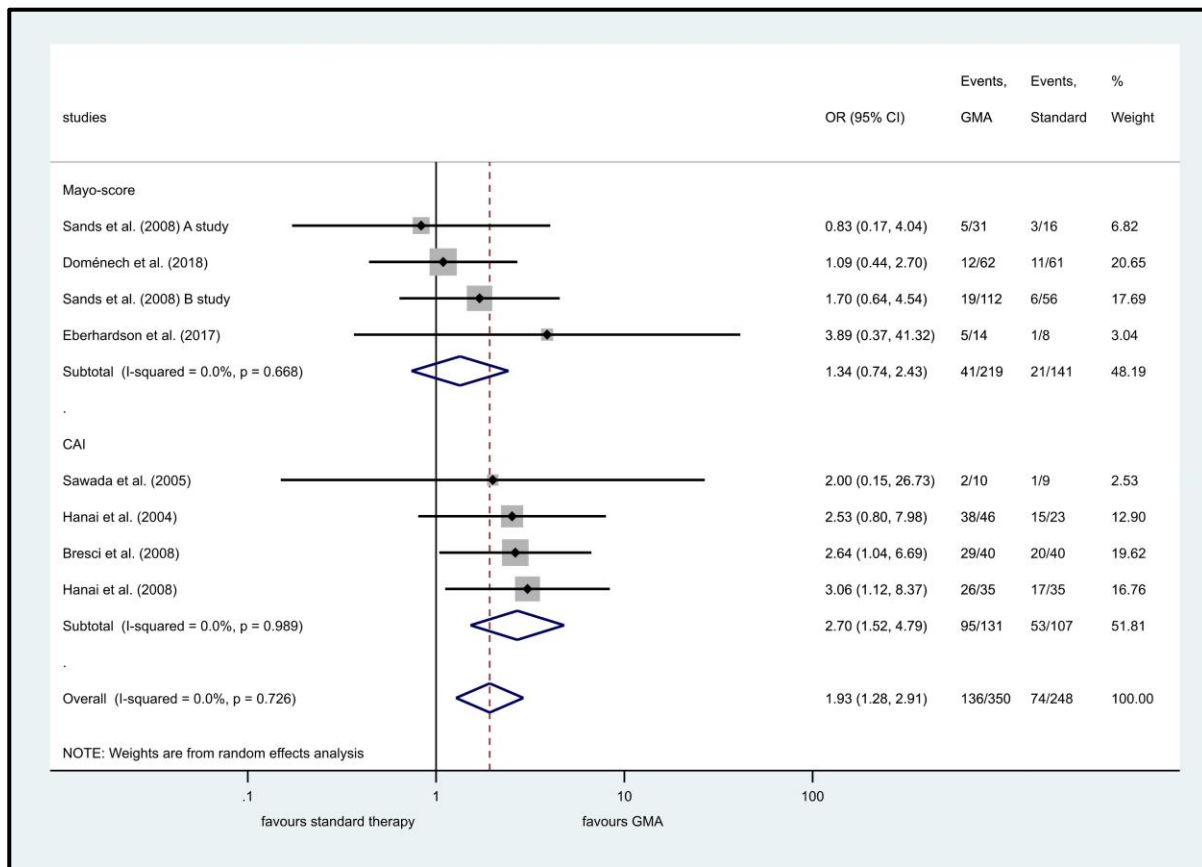
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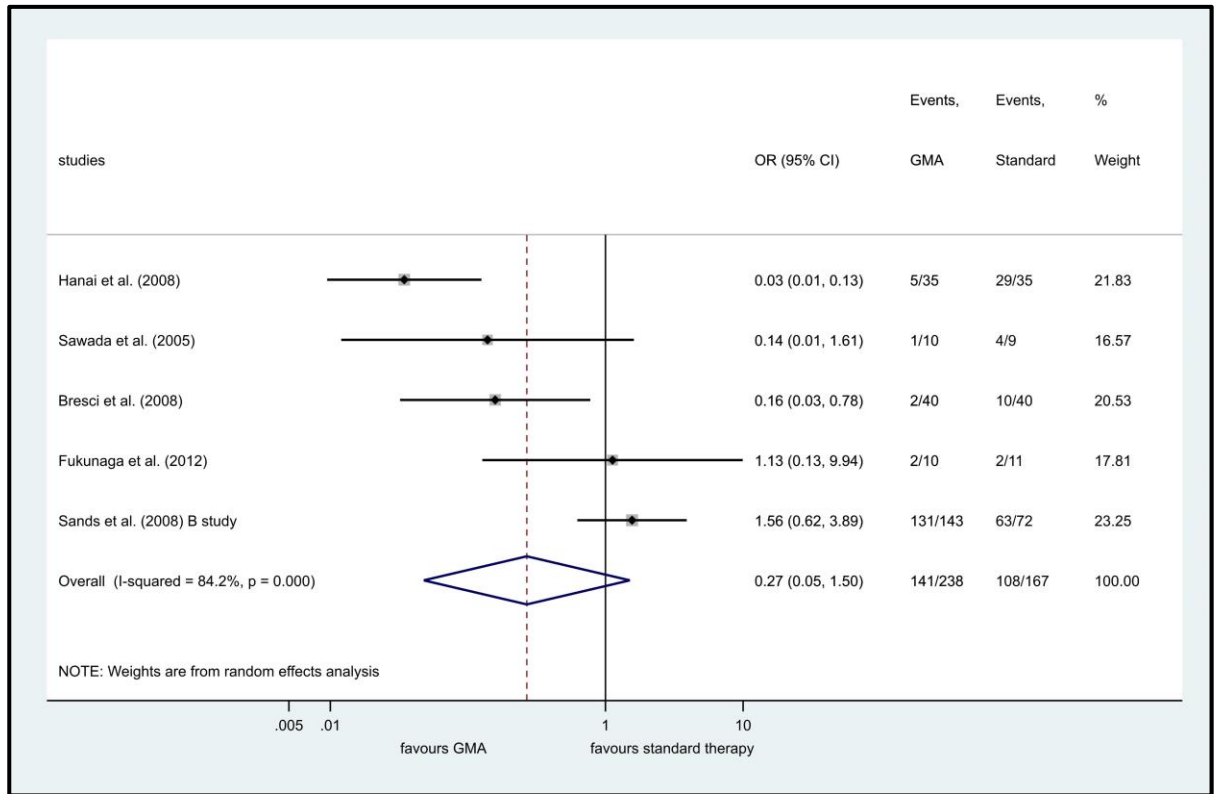




Supplementary Figure 5

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Supplementary Figure 7

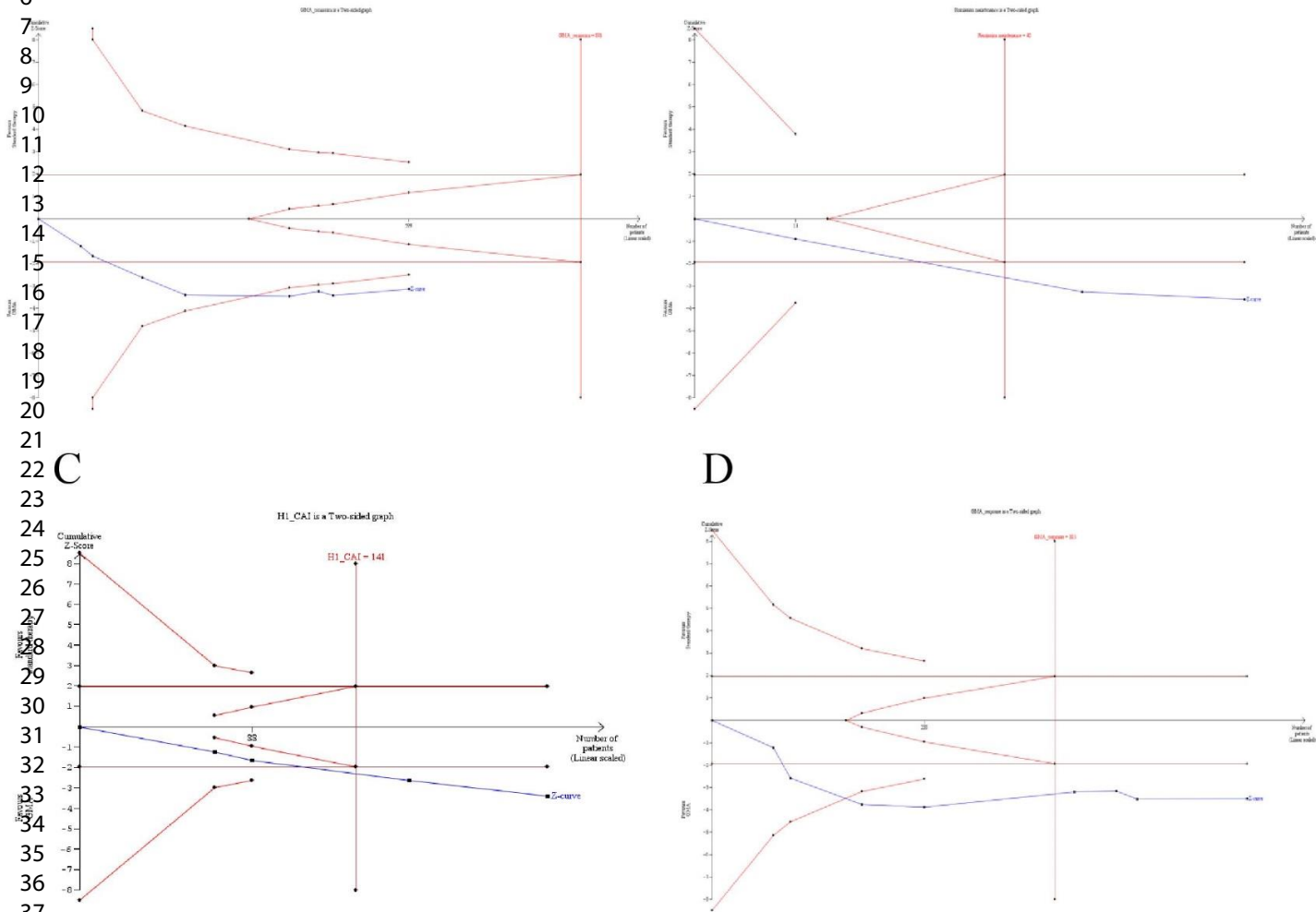
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Study	Reported adverse events
Hanai et al. 2004	flushing, nausea, mild fever
Sawada et al. 2005	fever, skin rash, back pain
Bresci et al. 2008	headache, gastrointestinal intolerance, facies lunaris, vascular hypertension, glucose intolerance
Fukunaga 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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