Granulocytapheresis in steroid-dependent and steroid-resistant patients with inflammatory bowel disease: A prospective observational study☆

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Abstract

Background: Despite the mounting importance of granulocytapheresis (GCAP) for inflammatory bowel disease (IBD) treatment, its effectiveness in steroid-dependent (SD) and steroid-resistant (SR) patients has not been clearly evaluated. This prospective observational study describes the use of GCAP in SD and SR patients with either Ulcerative Colitis (UC) or Crohn’s Disease (CD).

Methods: 118 patients, 83 affected by UC (55 SD and 28 SR) and 35 by CD (22 SD and 13 SR), were treated with GCAP, using Adacolumn™, for 5 consecutive weeks, 1 session/week. All patients were followed for 12 months after the end of GCAP. Clinical remission was defined as Clinical Activity Index (CAI) ≤ 6 for UC patients and Crohn’s Disease Activity Index (CDAI) < 150 for CD patients.

Results: All patients completed the study; no major complications were reported. At the end of GCAP 71% of UC and 63% of CD patients showed clinical remission. At 6 months the remission was maintained by 66% and 54% of UC and CD patients respectively, while at 12 months the percentages were 48% and 43%, respectively. No differences between SD and SR subgroups were reported at any timepoint. CAI and CDAI values significantly dropped after GCAP treatment and at 6 and 12 months follow-up (p < 0.05 vs baseline for both timepoints). No differences were measured in CAI and CDAI between SD and SR patients.

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1. Introduction

For many years oral and topical corticosteroids have been the cornerstone of treatment for moderate to severe Ulcerative Colitis (UC) and Crohn’s Disease (CD), proving their efficacy in induction of remission in up to nearly 60% of patients. The use of steroids poses however many challenges; although they offer an initial good response rate they are ineffective as maintenance therapy and frequently cause side effects.

Steroid treatment is usually required in large doses and may show different patterns of response in different patients. Up to 40% of patients do not respond to high dose steroid therapy and are therefore defined as steroid-resistant; in particular it has been calculated that approximately 22% of UC patients and up to 20% of CD patients develop resistance. A similar percentage of patients initially benefit from steroid treatment but seem to relapse shortly after the end of treatment or after dose reduction. They are defined as steroid-dependent patients and they require increasing doses of steroids usually above 10 mg/day to maintain remission, thus increasing the risk of developing adverse events.

In order to overcome these issues, new therapies have been introduced in the therapeutic armamentarium for IBDs treatment. Mesalazine represents a safe and effective option in the treatment of mild-to-moderate UC, while its efficacy in treating CD is still debated. Immunomodulators such as azathioprine or 6-mercaptopurine are also largely prescribed, but they require long-term administration to exert full efficacy and are therefore unsuitable during the acute phases of the disease. Newer immunosuppressant drugs like cyclosporine A are effective therapeutic options, but are associated with a high discontinuation rate. Last, encouraging results have been obtained with new biological agents, such as infliximab or adalimumab, even if the long-term efficacy and safety-profile of these molecules need further evaluation.

Given the insufficiency of current treatments in achieving a satisfactory control of IBDs, new therapeutic approaches are needed to overcome the above-mentioned limitations and particularly to help the condition of patients unresponsive or dependent to steroids.

In recent years some trials have introduced leuko- cytapheresis and granulocytapheresis (GCAP) as potential alternative treatments for IBDs. In particular GCAP, an apheresis technique based on the selective sequestration of granulocyte and monocyte subpopulations, has shown in several open trials on IBD patients efficacy rates up to 65–75% associated with an excellent safety profile. However the effectiveness of this therapy has been studied mainly on UC patients, while it requires further evaluation in patients affected by CD. Moreover its effectiveness in steroid-dependent and steroid-refractory patients has been poorly explored to date.

2. Patients and methods

2.1. Study setting and design

This was a prospective, observational study conducted at the Department of Gastroenterology of the Cisanello Pisa University Hospital (Pisa, Italy), a reference Center for the treatment of IBDs in Italy. The study protocol conformed to the ethical guidelines of the 2008 Declaration of Helsinki and was approved by the local ethical committee. Written informed consent was obtained before GCAP from all patients. Forty of the patients included in the present study had already been included in our previous studies on CD and UC.

2.2. Patients

Patients eligible to this study were steroid-resistant or steroid-dependent adult patients who suffered from UC (CAI > 6) or active CD (CDAI > 150). Steroid-resistance was defined as no disease remission (CDAI < 150 or CAI < 6) after the administration of methylprednisolone 1 mg/kg/day and of oral mesalazine 2.4 g/day during the 8 weeks prior to GCAP initiation. Steroid-dependence was instead defined as clinical response maintained only with steroid dosages >10 mg/day and oral mesalazine 2.4 g/day during the 8 weeks prior to GCAP initiation.

The time interval of 8 weeks was selected in order to evaluate the response to first-line steroids for a longer period than that usually considered in the standard definition of refractory patient (i.e., 4 weeks), in line with our previous studies on CD and UC.

The following exclusion criteria were applied: pregnancy, allergy to heparin, serious cardiovascular diseases, extra-intestinal manifestations, strictureing or penetrating (fistulising) disease (as documented by magnetic resonance), perianal disease, and treatment with immunosuppressant drugs or biological agents.

2.3. Study procedures

All included patients received a 5-session treatment (1 session/week, for 5 consecutive weeks) of GCAP. This is an extracorporeal procedure in which 1.8 L of blood is filtered through an
Adacolumn™ (JIMRO, Takasaki, Japan). This apheresis column, which has a capacity of 335 mL, contains nearly 35,000 specially designed cellulose diacetate beads, each with an average diameter of 2 mm, which bind to the CR3 receptors displayed on granulocytes and monocytes, thus resulting in the selective absorption of these cells from the peripheral blood. Each apheresis procedure requires the addition of 1,500 UI of sodium heparin as anticoagulant. Blood is obtained by antecubital vein puncture.

The daily dosage of methylprednisolone was progressively reduced over a 6-week period until complete discontinuation (one week after the last GCAP procedure) to avoid alterations to the hypothalamic–pituitary–adrenal axis. Patients who were on mesalazine were allowed to continue with this medication, but no additional treatment, such as immunosuppressants and/or anti-TNF agents, was permitted.

2.4. Study evaluations

The activity of the disease was evaluated by CDAI for CD patients and CAI for UC patients. Both indexes are widely used parameters to assess disease activity in clinical trials. A clinical remission was defined as CAI ≤ 6 or CDAI < 150. All visits were performed by a trained clinician. The incidence of clinical remission and the course of CAI and CDAI were evaluated over the follow-up period at 6 and 12 months.

All assessments were performed separately in patients with UC and in those with CD; we considered both the overall population of patients with each disease, and the stratified data according to steroid-resistance and steroid-dependence.

CAI and CDAI were calculated at baseline, after the end of the five-week course of GCAP, and at 6 and 12 months’ follow-up. The incidence of remission was assessed at the end of GCAP and at both follow-up timepoints. Patients who experienced disease relapse were excluded from the study and were re-treated with a further cycle of GCAP or other treatments, as appropriate. At baseline and at 12 months, we measured the values of some markers of clinical activity, namely ESR, CRP and calprotectin. The incidence of mucosal healing – as evaluated by endoscopy – was also assessed at 6 and 12 months.

2.5. Statistical analysis

All data were analyzed by descriptive statistics. Data from patients who relapsed and underwent further GCAP cycles were considered only until the need for a further re-treatment. The homogeneity at baseline of steroid-resistant and steroid-dependent populations in each disease group, with respect to age, gender, CAI and CDAI was evaluated using Student’s t-test, Chi-square test or Wilcoxon Rank Sum test, where appropriate. Differences in CAI and CDAI over time were assessed by ANOVA test for repeated measures, while differences in the remission rates at 6- and 12-month follow-up versus at the end of GCAP treatment and those between SD and SR subgroups for each disease were assessed by Chi-square test. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software for Windows.

Differences in remission rates between UC and CD patients were not evaluated as the differential efficacy of GCAP treatment in these two groups of patients has already been analyzed in a previous study which documented no significant differences in this outcome between UC and CD patients.

3. Results

Baseline characteristics of patients are summarized in Table 1. The whole group of patients and the subgroups of steroid-dependent and steroid-refractory patients were homogenous for age, gender, CDAI and CAI. Treatment with steroid was interrupted 3 weeks (median value; range: 2–4) before the initiation of GCAP, in both SR and SR patients.

No major complications were reported during the study. Only mild headache and fatigue were reported in 7% and 5% of patients, respectively.

Remission rates for UC and CD patients at the end of treatment and at 6 and 12 months' follow-up are summarized in Table 2, while in Table 3 the values of CDAI and CAI registered at baseline, at the end of GCAP treatment and at the two follow-up timepoints are reported.

3.1. Induction and maintenance of remission in UC patients

At the end of GCAP, 59 (71%) out of 83 UC patients showed a clinical remission of the disease, with remission rates of 73% for SD patients and 68% for SR patients. Six months after the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>UC</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number patients</td>
<td>118</td>
<td>83</td>
<td>35</td>
</tr>
<tr>
<td>Steroid-dependent</td>
<td>77</td>
<td>55</td>
<td>22</td>
</tr>
<tr>
<td>Steroid-refractory</td>
<td>41</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>38.4 ± 7.9</td>
<td>39.2 ± 8.6</td>
<td>37.7 ± 7.2</td>
</tr>
<tr>
<td>Male/female</td>
<td>67/51</td>
<td>48/35</td>
<td>19/16</td>
</tr>
<tr>
<td>Smokers</td>
<td>31</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>Previous surgical therapy</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extra-intestinal complications</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Disease duration (years ± SD)</td>
<td>7.1 ± 4.6</td>
<td>6.9 ± 5.7</td>
<td>7.4 ± 3.5</td>
</tr>
<tr>
<td>Months of remission before study entry</td>
<td>5.8 ± 4.2</td>
<td>6.0 ± 3.5</td>
<td>5.7 ± 4.9</td>
</tr>
<tr>
<td>Presence of granulomas</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>CAI (mean ± SD)</td>
<td>–</td>
<td>9.5 ± 2.4</td>
<td>–</td>
</tr>
<tr>
<td>CDAI (mean ± SD)</td>
<td>–</td>
<td>247 ± 37</td>
<td>–</td>
</tr>
<tr>
<td>ESR (mm/h) (mean ± SD)</td>
<td>85 ± 9</td>
<td>84 ± 8</td>
<td>86 ± 10</td>
</tr>
<tr>
<td>CRP (mg/dL) (mean ± SD)</td>
<td>36 ± 11</td>
<td>37 ± 13</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>Calprotectin (mg/kg) (mean ± SD)</td>
<td>240 ± 172</td>
<td>193 ± 143</td>
<td>322 ± 227</td>
</tr>
</tbody>
</table>

CAI: Clinical Activity Index; CDAI: Crohn’s Disease Activity Index; SD: Standard deviation.
The use of GCAP in clinical practice is increasing in recent years, thanks to the overall favorable results collected so far. Overall, 1 year after the treatment 48% of patients affected by UC were still in remission.

The mean value of CAI was significantly reduced from 9.5 ± 2.4 at baseline to 3.0 ± 3.1 (p < 0.05) at the end of GCAP treatment. The reduction in CAI was maintained at 6 months (3.3 ± 3.9; p < 0.05 vs baseline) and 12 months' (4.3 ± 3.4; p < 0.05 vs baseline) follow-up. No differences were reported between the SD and SR subgroups at any timepoint.

At 12 months, in responding patients the concentration of the markers of clinical activity was significantly decreased when compared with baseline values (ESR: from 84 ± 8 to 26 ± 7 mm/h; CRP: from 35 ± 11 to 0.4 ± 0.6 mg/dL; calprotectin: from 193 ± 143 to 37 ± 20 mg/kg; p < 0.05 for all comparisons). No significant differences were disclosed between the SD and SR subgroups.

The incidence of mucosal healing at 6 months was 58% in the overall population, 62% in SD patients and 56% in SR patients; at 12 months, the proportions of patients still with mucosal healing were 40%, 45% and 32%, respectively (p < 0.05 vs 6 months for all comparisons).

### 3.2. Induction and maintenance of remission in CD patients

Among patients with CD, 14 (63%) out of 22 SD and 8 (61%) out of 13 SR patients achieved clinical remission after GCAP, for an overall remission rate of 63% in the whole population. At 6 months' follow-up, 12 (55%) SD and 7 (54%) SR patients were still in remission, respectively, while 12 months after the end of the treatment the number of patients who had maintained remission were 10 (45%) and 5 (38%), respectively. Remission rates over time were not statistically different from those at the end of GCAP treatment and no statistical differences were reported between the SD and SR subgroups at any timepoint.

The mean CDAI significantly decreased from 247 ± 37 at baseline to 120 ± 49 (p < 0.05) at the end of GCAP treatment. At 6 months' follow-up the mean CDAI was 130 ± 51 (p < 0.05 vs baseline), with no differences observed between SR and SD patients, while at the end of the observation period its value was 140 ± 35 (p < 0.05 vs baseline), with a slightly more evident decrease – although statistical significance was not reached – in SR patients (133 ± 36) than in SD subjects (145 ± 42).

At 12 months, the concentration of the markers of clinical activity was significantly decreased in responding patients, when compared with baseline values (ESR: from 86 ± 10 to 25 ± 8 mm/h; CRP: from 37 ± 13 to 0.4 ± 0.4 mg/dL; calprotectin: from 322 ± 227 to 43 ± 24 mg/kg; p < 0.05 for all comparisons). No significant differences were disclosed between the two subgroups.

The incidence of mucosal healing was 48% in the overall population, 50% in SD patients and 47% in SR patients; at 12 months, the proportions of patients still with mucosal healing were 37%, 41% and 30%, respectively (p < 0.05 vs 6 months for all comparisons).

### 4. Discussion

The use of GCAP in clinical practice is increasing in recent years, thanks to the overall favorable results collected so far, as demonstrated by the reduced severity of disease activity and the increased rates of clinical and mucosal remission observed in both SD and SR patients.
Recent studies evaluated the efficacy of GCAP in patients with either UC or CD, and suggested that this technique can be effective in treating and maintaining the remission in IBD patients, while providing a more favorable safety profile if compared with other treatments. Nevertheless only few studies analyzed the differential efficacy of GCAP in patients who developed steroid-dependence or resistance, conditions which are quite common among patients who receive the first-line corticosteroid treatment; such studies included an overall limited number of patients and were all conducted in a population of only SD patients with UC in the wide majority of cases. Doménech et al. conducted a pilot study in 26 SD patients (14 with UC and 12 with CD) who were started on 60 mg/day of prednisone; after 7 days, a five-session GCAP was started. Remission rate was 62% and 70% in patients with UC and CD, respectively; at a median follow-up of 12.6 months, 6 UC patients and only 1 CD patient were still on clinical remission. Another study enrolled 18 SD patients with UC, and evaluated clinical remission, endoscopic remission and the relapse rate over a 1-year follow-up. Clinical remission was observed in 55% of patients, and endoscopic remission in 50%. The rate of remission and relapse over the follow-up period was 17%. Overall similar results were observed in a larger database study on 142 SD patients with UC, conducted in Spain. Importantly, an open-label randomized study compared the clinical outcomes of 96 patients with UC treated with 5 GCAP treatments with those reported in 90 patients with the same disease who received 10 GCAP sessions. A total of 196 adults with moderate–severe UC were randomized 1:1 to 5 (n = 82) or 10 (n = 80) open label apheresis treatments. Clinical Activity Index score improved from baseline in both groups (from 8.7 to 5.6 with 5 treatments, and from 8.8 to 5.4 with 10), and no significant differences between groups were reported. Similarly, the rates of clinical remission and clinical response were comparable between groups (clinical remission: 44% and 40%, respectively; clinical response: 56% and 59%, respectively).

The results of the previous studies in SD patients are overall in line with those reported in ours. However, our study provides, for the first time to our knowledge, a comprehensive evaluation of GCAP feasibility and tolerability in a large number of IBD patients who failed to respond or were dependent to steroids therapy. In particular it must be noticed that we included patients who had been refractory or dependent to steroids for a longer period of time than that usually considered in clinical practice to define lack of response.

To date, only few studies have investigated the efficacy and safety of GCAP in treating CD, and the results of the present study, which broadly evaluates not only UC but also CD patients, further confirm the findings of previous analysis on the efficacy of GCAP treatment in inducing and maintaining remission in a high proportion of CD patients. Moreover, the results also suggest that the efficacy of this therapy is maintained over time, as after GCAP treatment a relevant proportion of patients (43%) is still in remission 12 months after the procedure.

Importantly, the results of the present study suggest that the tolerability and efficacy of GCAP are not limited to SR patients, as previously reported, but can be observed also in SD patients with clinically-relevant benefits: 63% of responders after the treatment and 45% of patients still in remission at 12 months' follow-up, and both CDAI values were significantly lower at both 6 and 12 months than at baseline. The improvement in disease symptoms experienced by both SD and SR patients is paralleled by the reduction in the concentration of markers of clinical activity reported in responding patients of both subgroups. The trend of clinical response was also mirrored by that of mucosal healing: A high proportion of patients presented with mucosal healing at 6 months after the end of GCAP. Benefits in terms of mucosal healing were still observed at 12 months, although the proportion of patients with this outcome was significantly lower than that reported at 6 months. These results may be of clinical relevance as they suggest GCAP as a valid alternative treatment for patients affected by CD who are either refractory or dependent on corticosteroid treatment.

This study also supports the results of previous analysis on the use of GCAP in the treatment of patients with UC. Our findings show that nearly 71% of UC patients treated with GCAP achieved clinical remission, with very similar remission rates among SD (73%) and SR (68%) patients; CDAI values were significantly lower at both 6 and 12 months than at baseline and remission rates were maintained over time. Moreover, we registered no differences between SR and SD patients in terms of remission rates and CDAI values at 6 and 12 months' follow-up. Several studies evaluated the importance of GCAP among UC patients; however to our knowledge only one study has evaluated patients who are either refractory or dependent to steroids. Our results are in line with previous evidence, but are obtained in an almost doubled number of patients; in addition the analysis was carried out not only at the end of GCAP treatment, but patients were followed for up to 12 months in order to evaluate the long-term effectiveness and tolerability of GCAP.

Some limitations of this study must however be acknowledged. First of all this is a prospective observational study which lacks the rigor and high validity of randomized control trials, which appear therefore necessary to further confirm our findings. However, the inherent limitations of non-randomized, uncontrolled studies, such as the presence of confounding variables and the high variability of patient traits, are counterbalanced by the broader applicability of observational real-life data on patients in clinical settings. Another limitation to this study is the lack of a direct comparator. We cannot precisely determine if the results obtained in this study are directly linked to GCAP therapy or are instead due to the natural course of the disease, nor can we establish the extent of the placebo effect.

Despite these limitations, our findings, collected in a rather large sample of patients, suggest that GCAP is safe and effective in inducing and maintaining remission not only in UC but also in CD patients. Moreover this study shows that there is no difference in GCAP efficacy between SD and SR patients, therefore suggesting that this therapy can represent an effective and safe option in this population. Randomized controlled trials are however necessary to further prove the efficacy and safety of this technique on these patients.
Conflict of interest

The authors have no conflicts of interest directly relevant to this study.

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References