ORIGINAL ARTICLE

Feasibility and safety of granulocytapheresis in Crohn’s disease: A prospective cohort study

Faisabilité et sécurité des granulocytaphérèse dans la maladie de Crohn : une étude prospective de cohorte

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Summary
Background and objective. — This study evaluated the feasibility and safety of granulocytapheresis (GCAP) in inducing and maintaining remission in refractory Crohn’s disease. The relationship between the clinical outcomes and the location (ileal or ileocolonic) of disease was also assessed.

Patients. — We evaluated 16 patients with ileal location (group A), 14 with ileocolonic location (group B). The patients underwent five sessions (1 session/wk) of GCAP (Adacolumn™). CDAI was measured at the end of GCAP, at 6, 9 and 12 months.

Results and conclusions. — No major complications were observed. At the end of GCAP, 19 (63.3%) patients showed a clinical remission: 10 (62.5%) in group A versus 9 (64.2%) in group B. At 6 months, 16 (53.3%) of the cases had maintained remission: 9 (56.2%) in group A versus 7 (50.0%) in group B. At 9 months, 13 (43.3%) patients had maintained remission: 7 (43.7%) in group A versus 6 (42.8%) in group B. At 12 months, 12 (40%) patients were still in clinical remission: 7 (43.7%) in group A versus 5 (35.7%) in group B. Risk of relapse was not related to disease location. The procedure was well tolerated and feasible in an important percentage of Crohn’s disease patients.

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Introduction

The definition "inflammatory bowel diseases (IBD)" usually includes two similar but distinct chronic diseases of the gut,
ulcerative colitis and Crohn’s disease, that are both characterised by episodes of remission and exacerbation with systemic complications [1—4].

During the last 20 years, the treatment of IBD has greatly improved; however, it is still empirical, due to the unknown aetiology of such diseases [5—8]. Both ulcerative colitis and Crohn’s disease are supposed to have a multifactorial aetiology; however, regardless the cause, the final pathway leading to tissue damage in IBDs is mediated by the cellular immune response, through white blood cells, in the intestinal mucosa.

Corticosteroids are a mainstay of acute therapy for moderate to severe ulcerative colitis or Crohn’s disease; however, up to 40% of patients do not respond to high-dose steroid therapy [9]. Preliminary results suggested that aminosalicylates, e.g. mesalazine, can be safe in the treatment of active Crohn’s disease, but the clinical efficacy of mesalazine is currently under debate [9]. Classic immunosuppressant drugs, such as azathioprine or mercaptopurine, need some weeks to exert their full activity, and are therefore of limited use during the acute phases of the diseases. Newer immunosuppressants like cyclosporine and/or biological agents (e.g. anti-Tumor Necrosis Factor agents) have become available in the therapeutic armamentarium for the treatment of IBD. Unfortunately, many patients show often serious side effects after the administration of these drugs or are unresponsive to these medications [10].

Therefore, new therapeutic approaches are needed to improve the clinical outcome in active steroid-refractory IBDs. In recent years, some trials have suggested that granulocytapheresis (GCAP), a technique that selectively sequestrates granulocyte and monocyte subpopulations, can be a useful and safe option to induce clinical remission in patients with IBD [11—16]. However, these studies mainly involved patients affected from ulcerative colitis, while fewer data are currently available on the use of GCAP in the treatment of patients with Crohn’s disease.

In the present study, we report our experience using GCAP when treating patients with Crohn’s disease who failed to respond to conventional treatment.

The primary objective of this trial was to evaluate the safety and feasibility of GCAP in inducing and maintaining remission in patients with Crohn’s disease who were refractory to conventional treatment with steroids and mesalazine. A secondary objective was to assess a possible relationship between the efficacy of GCAP and the location (ileal or ileocolonic) of the disease.

Patients and methods

The study protocol conformed to the ethical guidelines of the 2008 Declaration of Helsinki and was approved by the Ethical Committee of our hospital. Written informed consents were obtained before GCAP from all patients.

Consecutive patients with active Crohn’s disease (Crohn’s Disease Activity Index [CDAI] > 150) who were refractory to steroid and mesalazine therapy were eligible to this study. These subjects were defined as refractory if they did not achieve disease remission (CDAI ≤150) after the administration of methylprednisolone 1 mg/kg/day and oral mesalazine 2.4 g/day, during the 8 weeks prior to GCAP initiation. We selected this time period in order to evaluate the response to first-line steroids for a longer period than the one usually considered in the standard definition of refractory patient (i.e. 4 weeks).

Exclusion criteria were: pregnancy, allergy to heparin, serious cardiovascular diseases, extraintestinal manifestations, structuring or penetrating (fistulising) disease, perianal disease, actual treatment with immunosuppressant drugs or biological therapy, steroid-dependence.

The activity of the disease was evaluated by CDAI, the main index of disease activity used in clinical trials [17,18].

From September 1st 2005 to December 31st 2009 we have identified 30 consecutive patients with active Crohn’s disease who were refractory to steroid therapy and were visited at our Center. These patients were divided in two groups according to disease location: group A, 16 patients, with ileal location of disease, while group B, 14 patients, with ileocolonic location of disease. Patients were classified

Table 1 Characteristics of patients at baseline.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>A</th>
<th>B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number patients</td>
<td>30</td>
<td>16</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>36.5 ± 5</td>
<td>36 ± 6</td>
<td>37 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Male/Female</td>
<td>17/13</td>
<td>9/7</td>
<td>8/6</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Previous surgical therapy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Extraintestinal complications</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration(years ± SD)</td>
<td>6.5 ± 3.5</td>
<td>7.0 ± 4.0</td>
<td>6.0 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Months of remission before study entry</td>
<td>4.5 ± 2.0</td>
<td>5.0 ± 2.0</td>
<td>4.0 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Steroids use (days ± SD)</td>
<td>58</td>
<td>56 ± 2.0</td>
<td>56 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Non-stricturing/non penetrating</td>
<td>30</td>
<td>16</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Presence of granulomas</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>CDAI (mean ± SD)</td>
<td>235 ± 35</td>
<td>240 ± 30</td>
<td>230 ± 40</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mean ± SD)</td>
<td>35 ± 15</td>
<td>40 ± 20</td>
<td>30 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>ESR (mean ± SD)</td>
<td>85 ± 9</td>
<td>80 ± 10</td>
<td>90 ± 8</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not significant. SD: standard deviation.
Adacolumn™ is a 335 mL capacity column filled with 35,000 cellulose diacetate beads (2 mm in diameter) that binds granulocytes and monocytes via the CR3 receptors displayed on these cells. Each apheresis procedure requires the addition of 1,500 UI of sodium heparine as an anticoagulant. Blood is collected by puncturing the antecubital vein.

The daily dosage of methylprednisolone was progressively tapered over a 6-week until discontinuation (one week after the last GCAP procedure) to avoid alterations to the hypothalamic-pituitary-adrenal axis, while the treatment with mesalazine 2.4 g/day was maintained during the treatment and follow-up.

After the end of the five-week course of GCAP and every 6, 9 and 12 month, each patient was visited for a clinical assessment and CDAI was evaluated.

The primary outcome measure was the proportion of patients achieving remission (CDAI < 150). Responding patients were followed for 12 months from the last session of GCAP to evaluate the incidence of relapse (defined as CDAI > 150). Patients who were on mesalazine continued with this medication but no additional treatment (e.g. immunosuppressants and/or anti-TNF agents) was permitted. Clinical laboratory values (CPR and ESR) were recorded before, during and after GCAP.

Statistical analysis was performed using SPPS software for Windows. The homogeneity of two groups with respect to age, gender, CDAI, CPR and ESR was evaluated at the outset of the study using Student’s t, Chi-square test or Wilcoxon Rank Sum test, where appropriate. Cumulative relapse rates were evaluated at the end of the study by the log rank test and 95% confidence interval. Factors associated with remission were investigated by logistic regression model. A value of $p < 0.05$ was considered statistically significant for all the tests.

### Results

Patients with ileal or with ileocolonic location of disease were homogeneous for age, gender, CDAI, CPR and ESR (Table 1). No major complications occurred during the five-week course of GCAP only a transient mild headache was reported in four (13.3%) patients.

Rates of remission at different timepoints, for the overall sample, are summarized in Table 2. One week after the end of GCAP, 19 (63.3%) out of 30 patients showed a clinical remission of the disease. In these patients, mean CDAI decreased from 235 ± 35 to 129 ± 15, mean CRP values decreased from 35 ± 15 to 11 ± 5 mg/L ($p < 0.05$), and mean ESR values from 85 ± 9 to 25 ± 6 mm/h ($p < 0.05$ for all comparisons). At the end of the observation period, the mean value of CRP was 0.4 ± 0.4 in responding patients and 32 ± 13 in non-responders. Six months after the end of the treatment, 16 (53.3%) patients were still in remission; 13 (43.3%) and 12 (40.0%) patients had maintained remission at 9 and 12 months from GCAP, respectively.

No differences were disclosed in the rate of remission between the two groups and after the stratification according to disease location (secondary analysis) (Table 2). Moreover, statistical analyses revealed that the risk of relapse is not related to the location of the disease. Cumulative relapse rates are depicted in Fig. 1.

### Discussion

Corticosteroids and mesalazine are considered first-line therapies in the management of Crohn’s disease [20,21]. More effective drugs, such as azathioprine, mercaptopurine, cyclosporine and/or biological agents may be introduced if disease activity persists despite the administration of first-line treatment. However, a patient may be considered refractory to first-line drugs only after having carefully considered if initial therapy has been administered at adequate doses and for appropriate duration of treatment.

Large doses of steroids are often required to control active disease and some patients do not respond to this conventional treatment. However, steroid treatment may show different patterns of response in different patients. For example, some patients may experience favorable clinical outcome after the administration of this therapy, while other subjects may show an initial response, but lose benefit as treatment is interrupted. Other patients do not show any clinical improvement despite high-dose or prolonged steroid

<table>
<thead>
<tr>
<th>Table 2 Rates of remission at different timepoints.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>End of treatment</td>
</tr>
<tr>
<td>6 months</td>
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<tr>
<td>9 months</td>
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<tr>
<td>12 months</td>
</tr>
</tbody>
</table>

NS: not significant.
therapy. Of note, colectomy rates vary from 10% to 40% in these patients [10]. Patients are usually classified as non-responders if disease activity still persists at 4 weeks from the initiation of steroid treatment. In our study, we decided to continue the treatment for further 4 weeks, in order to evaluate the response to first-line steroids for a longer period.

On the other hand, about 15% of patients treated with mesalazine experience intolerance to this drug; moreover, the efficacy of mesalazine is still being questioned, especially in patients with ileal Crohn’s disease [9].

In refractory patients, treatment with cyclosporin was shown to avoid acute colectomy in 57% of cases, but after a 6-month follow-up 73% of patients eventually underwent surgery [22]. Azathioprine or 6-Mercaptopurine have been reported to be beneficial in steroid resistant cases, but they require a long-term administration to exert their full efficacy [23]. Furthermore, a significant number of patients present serious side effects or are unresponsive to newer immunosuppressants like anti-TNF agents [10].

Inflammatory bowel diseases are associated with elevated circulating and tissue levels of leukocytes [24]. In particular, granulocytes are the first cells mobilized to inflammation sites, where they interact with lymphocytes to orchestrate the inflammatory response [25–28]. For these reasons, the removal of granulocytes may be a logical therapeutic manoeuvre. GCAP acts on specific subpopulations of leukocytes which play a key role in the inflammatory process. Recent data from literature have pointed out that this technique can represent an effective treatment in patients with ulcerative colitis or Crohn’s disease, possibly with a more favourable safety profile when compared with other treatments [11–14]. The good results displayed by GCAP have been attributed not only to the removal of granulocytes and monocytes, but also to an immunomodulatory effect [11–14]. However, evidence supporting the effectiveness of GCAP in patients affected from Crohn’s disease is still quite scant.

In our patients, the procedure was well tolerated and feasible in an important percentage of Crohn’s disease patients especially in the first six months after GCAP. At one year after the GCAP, a rather relevant (40%) proportion of patients were still in remission. This finding may have some clinical importance, as the study population included only patients who had failed to respond to conventional therapies.

Our study provides, for the first time to our knowledge, a description of the feasibility and safety of GCAP in patients with Crohn’s disease who were refractory to steroid therapy. In order to limit the incidence of confounding factors (e.g. the presence of stricturing or penetrating disease, steroid-dependence, use of immunosuppressants and/or biological drugs) we applied strict exclusion criteria, that resulted in a long period of enrolment. Moreover, some patients were reluctant to provide consent for GCAP, as the use and knowledge of this technique are limited in Europe, especially when in comparison to other regions (e.g. Japan). However, the application of rigid inclusion criteria decision allowed for a robust evaluation of the feasibility and safety of GCAP in patients who were refractory to steroid treatment for a longer period of time than that usually considered in clinical practice to define the lack of response. We must acknowledge, however, that while we compared responders and non-responders to GCAP in terms of CRP, while we did not perform a similar comparison for some important clinical parameters (e.g. steroid requirement). Such comparison will be the subject of a future ad hoc study.

Of note, the relapse rate was comparable in patients with ileal or ileo-cecal location of disease. The lack of differences between subjects who presented either location of disease at baseline in all evaluated characteristics may strengthen the relevance of this finding, as no this result may not be affected by selection bias.

GCAP was well tolerated: no major adverse events were reported. This finding may be of clinical relevance, as other therapies used in refractory patients, such as steroids, immunosuppressants and/or anti-TNF agents, may be associated with a on set of significant adverse events.

In conclusion, this study suggests that GCAP could be an important innovation and an useful therapy after the failure of conventional treatments in patients with Crohn’s disease. We believe that our data could represent a starting point for further larger studies, possibly of comparative nature or addressing different outcomes, aimed to evaluate the real usefulness of GCAP in this population, to clarify the optimal length of treatment and to identify which patients could obtain more benefit from GCAP.

Conflict of interest statement

No potential conflict of interest relevant to this article was reported.

References


