Journal of Crohn's and Colitis, 2016, 5–12
doi:10.1093/ecco-jcc/jjv126
Advance Access publication July 18, 2015
Original Article

Original Article

Prospective Evaluation of the Achievement of Mucosal Healing with Anti-TNF-α Therapy in a Paediatric Crohn's Disease Cohort

Federica Nuti, Fortunata Civitelli, Silvia Bloise, Salvatore Oliva, Marina Aloi, Giuseppe Latorre, Franca Viola, Salvatore Cucchiara

Pediatric Gastroenterology and Hepatology Unit, Sapienza University Rome, Italy

Corresponding author: Salvatore Cucchiara, MD, PhD, Department of Pediatrics, Pediatric Gastroenterology and Liver Unit, University Hospital Umberto I - Sapienza University of Rome, Viale Regina Elena 324 - 00161 Rome. Tel: 390649979326/9324/9269; fax: 390662277415; Email: salvatore.cucchiara@uniroma1.it

Abstract

Background and Aims: There is growing evidence that in Crohn's disease the achievement and maintenance of mucosal healing (MH) through anti-TNFα antibodies may change the natural history of the disease. Few studies evaluating such outcome as a therapeutic goal are available in paediatrics. The primary aim of the study was to assess the efficacy of biologics in obtaining MH in a paediatric Crohn's disease cohort. The secondary aims were: (1) to assess response based on early or late treatment introduction and on combination therapy with immunomodulators versus biologics alone; and (2) to evaluate clinical outcome 2 years after the second endoscopy.
Methods: Biologic-naive paediatric Crohn's disease patients starting anti-tumour necrosis factor α (TNFα) treatment were enrolled. Patients' demographic and treatment data were recorded. Clinical [Pediatric Crohn's Disease Activity Index (PCDAI)] and endoscopic [Simple Endoscopic Score for Crohn's Disease (SES-CD)] evaluations were performed at time 0 (T0) and after 9–12 months (follow-up). Appropriate induction and maintenance therapeutic schemes were applied.
Results: Thirty-seven patients were enrolled. At enrolment, mean age was 12.3 ± 3.4 years and mean disease duration was 13.0 ± 16 months. At follow-up there was a significant decrease in PCDAI and SES-CD compared with T0 ($p < 0.01$). No statistical difference in frequency of MH between the early and late treatment introduction groups was found. Combination therapy was superior in obtaining complete plus partial MH ($p < 0.01$). One and 2 years after the second endoscopy, all and 79% of patients with complete MH and 75 and 67% of those with partial MH were still in clinical remission, respectively.
Conclusions: Biologics improve mucosal lesions, apparently more effectively if given in combination with immunomodulators. MH appears to sustain a better disease course.

Key Words: Anti-TNFα agents; paediatric Crohn's disease; mucosal healing

1. Introduction

Crohn's disease (CD) is a chronic relapsing inflammation of the gut that primarily affects young individuals, often leading to significant impairment of quality of life. The incidence of this disease has been rising in people of paediatric age during recent decades and, to date, about 25% of patients are diagnosed under the age of 18. Moreover, epidemiological and observational studies are pointing out that this population of patients appears to have a more severe and disabling course of disease when compared with adult cohorts.[1–4]

Several efforts have been made to optimize the use of available therapies to improve patient outcome, but so far nothing seems capable of changing the natural history of the disease. Hence, the major objective of medical therapies in CD should be the modification of the clinical course of the disease by stopping disease progression, reducing surgeries, hospitalizations and treatments with corticosteroids, as well as promoting growth and pubertal development.[5,6]

Mucosal healing (MH) has recently been emphasized as a therapeutic goal able to predict sustained clinical remission.[7,8] Of the therapies available, immunomodulators, such as azathioprine, 6-mercaptopurine and methotrexate, enteral nutrition and anti-tumour necrosis factor α (TNFα) agents have been shown to promote MH, although the last have a more efficacious and rapid effect compared with immunomodulators.[8,9,10]

Studies on adult populations suggest that scheduled maintenance therapy with infliximab, a chimeric anti-TNFα antibody, is associated with MH in up to 68% of patients.[7,11,12] Moreover, the ACCENT I endoscopic substudy suggested that MH may be associated with fewer hospitalizations, surgeries and intensive care unit stay after a 1-year period of infliximab maintenance therapy.[12] Schnitzler et al.[11] evaluated the influence of MH under infliximab treatment on the course of CD in adult patients, showing that sustained clinical benefit was best achieved in patients receiving scheduled maintenance infliximab therapy who achieved MH.

Studies assessing the effect on MH of adalimumab, a completely humanized anti-TNFα antibody, showed a rate of MH of up to 44% following adalimumab therapy in adult populations with CD.[13,14]

Recently a new concept of remission, defined as deep remission, has been coined: it embraces MH plus clinical and/or laboratory marker normalization.[15] It was evaluated as the outcome in 252 inflammatory bowel disease (IBD) patients, of whom 183 had CD, treated with anti-TNFα antibodies; in this population 48% of patients achieved deep remission after a median of 23 months of maintenance therapy.[16]

Few studies have evaluated MH as a therapeutic goal and its influence on disease history in paediatric populations with CD.[17,18]

This single-centre prospective study aimed at assessing the effectiveness of therapy with anti-TNFα antagonists in obtaining MH in a paediatric cohort of CD patients. We also assessed the rates of MH based on early versus late introduction of anti-TNFα antagonists and on the concomitant use of immunomodulators and the influence of MH on patients' subsequent outcome.

2. Methods

2.1. Patients

We prospectively enrolled all patients with an established diagnosis of CD (age range 6–18 years) who started therapy with an anti-TNFα agent (infliximab or adalimumab) between January 2009 and October 2012. All were naive to biological therapies but could have been previously treated with corticosteroids, immunomodulators and aminosalycilates. Exclusion criteria were the need for immediate surgery, symptomatic stenosis or ileal or colonic strictures with prestenotic dilatation, and the presence of bacterial or viral infections.

2.2. Procedure

At baseline the following data were collected: age at diagnosis, indication for biologics, age at first administration, disease duration, disease location and phenotype according to the Montreal classification, and concomitant medications. Prior to the administration of biological therapy, all subjects were screened for acute or chronic infections by means of stool cultures, the Mantoux tuberculin skin test and the QuantiFERON test for tuberculosis, Epstein–Barr virus and cytomegalovirus serology and DNA polymerase chain reaction (PCR), hepatitis serology and vaccination history.

Ileo-colonoscopy and biopsies were performed in all patients before anti-TNFα therapy and after 9–12 months to evaluate MH.

Disease activity was assessed with the Pediatric Crohn's Disease Activity Index (PCDAI), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) at time 0 (T0) and at the time of endoscopic follow-up (T2) to assess the correlation between clinical and endoscopic appearance. Clinical response to treatment required a 15-point decrease in PCDAI score. Clinical remission was defined as the absence of symptoms related to CD and PCDAI ≤10. The normal ranges for CRP and ESR were considered to be 100–6000 μg/L and 0–20 mm/h, respectively. Serological remission was defined as a return to normal values.

Endoscopic activity was scored using the Simple Endoscopic Score for Crohn's Disease (SES-CD).[19] Complete MH was defined as a SES-CD of 0–1, meaning no signs of active inflammation of any colonic segment or in the terminal ileum; partial MH was defined as a reduction of 50% in SES-CD from baseline, no endoscopic healing and no variation or worsening of the SES-CD score. Endoscopy reading was performed by a single endoscopist (S.O.).

Patients received either infliximab or adalimumab with a regular induction treatment (5 mg/kg intravenously at 0, 2 and 6 weeks for infliximab and 160 mg, followed by 80 mg subcutaneously every other week for adalimumab), followed by maintenance therapy (5 mg/kg intravenously every 8 weeks for infliximab and 40 mg subcutaneously every other week for adalimumab). Concomitant immunomodulator/s and aminosalycilates were allowed and recorded. Concomitant treatment with immunomodulators was stable in all patients who had already been on immunomodulators for at least 3 months, except for patients who first received anti-TNFα treatment at diagnosis for whom the two treatments were introduced together.

The primary analysis assessed the proportion of patients achieving MH with maintenance therapy with biologics. The secondary analysis focused on differences in achieving MH according to concomitant use of immunomodulators and disease duration at first infusion; the early disease group comprised patients with a disease duration of less than 1 year prior to treatment introduction and the late disease group comprised those with a disease duration of more than 1 year prior to therapy introduction. Finally, the influence of MH on the further clinical course, in particular the clinical outcome of patients at a further follow-up of 12 months after the second endoscopy, was evaluated.

The study was approved by the ethics committee of the hospital and written informed consent was obtained from all enrolled children and their parents.

2.3. Statistical analysis

A descriptive analysis of the cohort was performed for the patients' baseline characteristics. The qualitative variables were described as the distribution of absolute frequencies and percentages, whilst the continuous variables were expressed as medians ± SD.

We composed frequency tables and plotted graphs of continuous and categorical variables. Differences between groups for continuous variables were tested using non-parametric tests, since the variables were not normally distributed. We used the χ^2 test to compare categorical variables and the Wilcoxon signed rank test for pair comparisons (T2 – T0). Spearman's correlation coefficient was used to test the correlation between continuous variables. Finally, a multivariate

logistic regression analysis was conducted using 'complete MH' or 'complete or partial MH' (two separate models) as dependent variables. The stepwise procedure (backward elimination) was used; in the first step we used all the variables that had a p value <0.20 in the univariate analysis. The results are presented as odds ratio (OR) and 95% confidence interval (CI).

Kaplan–Meier survival analysis was performed to evaluate differences according to therapy and endoscopic outcome at follow-up endoscopy in the clinical outcome a year after the second endoscopy. Differences between groups were assessed using log-rank and Breslow tests.

Statistical significance was set at $p < 0.05$. Statistical analysis was conducted using SPSS for Windows, release 19.0.

3. Results

3.1. Baseline characteristics

Thirty-seven patients were prospectively enrolled between January 2009 and October 2012. The main baseline characteristics are listed in Table 1.

Of the 37 patients, 25 (67.6%) were treated with infliximab and 12 (32.4%) with adalimumab. Disease duration prior to therapy was 14.5 ± 16.7 and 9.4 ± 14.5 months for infliximab and adalimumab patients, respectively, with 18 patients (9 infliximab, 9 adalimumab) having less than 1 year of disease history. Altogether, the indications for biological therapy introduction were as follows: unresponsiveness to conventional therapy, 46%; perianal disease, 27%; aggressive and extensive disease, 16%; and adverse events after immunomodulator use, 11%. The PCDAI and SES-CD at baseline were 31.3 ± 17.8 and 15.4 ± 8.2, respectively (infliximab, 31 ± 18.6 and 17 ± 9, respectively; adalimumab, 31.8 ± 17 and 12 ± 6, respectively). The proportion of patients on concomitant immunomodulators was 62% and the duration of such combinations was 8 ± 3.8 months (range 1–17 months).

3.2. Outcome of anti-TNFα treatment

At the follow-up endoscopy, 36% and 41.7% of patients on infliximab and adalimumab, respectively, presented complete MH, with an overall rate of 38%. The rate of partial MH was 36% in the infliximab group and 25% in the adalimumab group. The overall rate of partial MH was 32% and that of no MH was 30%. No statistical difference between the two therapies in achieving complete MH was found ($p = 0.74$). Moreover, considering complete MH plus partial MH as the outcome (70% of patients), again no statistical difference between the two therapies was found ($p = 0.74$).

Evaluating clinical outcomes, 67.5% of patients were in remission at the time of the follow-up endoscopy. All patients with complete MH were in clinical remission compared with 50% of those achieving partial MH.

The mean values of both PCDAI and SES-CD decreased significantly in all patients at follow-up ($p = 0.0001$). The values at follow-up were 9.6 ± 11.3 and 5.6 ± 7.1 respectively (Figures 1 and 2).

When considering deep remission, defined as clinical, endoscopic and serological remission, 35% of our patients were in deep remission at the time of the follow-up endoscopy.

A multivariate analysis was conducted to evaluate the association between patients' characteristics at baseline and their outcome at the follow-up endoscopy. Both complete MH and the combination of complete plus partial MH were considered as the outcome in this evaluation. A higher value of ESR at baseline appeared inversely correlated to both outcomes. The concomitant use of immunomodulators and a higher baseline value of SES-CD were positively correlated to the complete plus partial MH outcome. Older age at diagnosis was positively correlated to complete MH (Table 2). There was a positive correlation among the values of PCDAI, the inflammatory markers ESR and CRP, and SES-CD both at baseline and at follow-up.

3.3. Differences between early and late treatment

Dividing patients according to disease duration prior to treatment introduction, SES-CD values decreased significantly in both the early and the late disease group at follow-up ($p < 0.01$ and $p < 0.05$, respectively), and there was no statistical difference in achieving complete MH or complete plus partial MH between the two groups

Table 1. Patient characteristics at baseline.

Characteristic	Infliximab, 25 patients	Adalimumab, 12 patients	All 37 patients
Sex (male), *n* (%)	18 (72%)	5 (41.6%)	23 (62%)
Age at diagnosis (years), mean ± SD (range)	12.5 ± 3.8 (5.6–18.3)	12 ± 2.3 (8.4–15.5)	12.3 ± 3.4 (5.6–18.3)
Age at enrolment (years), mean ± SD (range)	13.4 ± 3.8 (6.6–18.3)	12.6 ± 2.7 (8.6–15.7)	13 ± 3.4 (6.6–18.3)
Disease duration at enrolment (months), mean ± SD (range)	14.5 ± 16.7 (0.5–63)	9.4 ± 14.6 (1–54)	13 ± 16 (0.5–63)
Disease location and behaviour	L1, 8%	L1, 41.6%	L1, 19%
	L2, 16%	L2, 0%	L2, 10.8%
	L3, 24%	L3, 16.7%	L3, 21.6%
	L4, 0%	L4, 0%	L4, 0%
	L1 + L4, 8%	L1 + L4, 16.7%	L1 + L4, 10.8%
	L2 + L4, 4%	L2 + L4, 0%	L2 + L4, 2.7%
	L3 + L4, 36%	L3 + L4, 25%	L3 + L4, 32.4%
	B1, 72%	B1, 75%	B1, 73%
	B2, 20%	B2, 25%	B2, 21.6%
	B3, 0%	B3, 8.4%	B3, 2.7%
	P, 40%	P, 16.7%	P, 32.4%
Previous therapies	IM, 68%	IM, 75%	IM, 70%
	Corticosteroids, 63%	Corticosteroids, 83%	Corticosteroids, 73%
	EEN, 37%	EEN, 17%	EEN, 32.4%

IM, immunomodulators; EEN, exclusive enteral nutrition.

Montreal classification for Crohn's disease: L1, ileal; L2, colonic; L3, ileocolonic; L4, upper gastrointestinal tract; B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating; P, perianal disease.

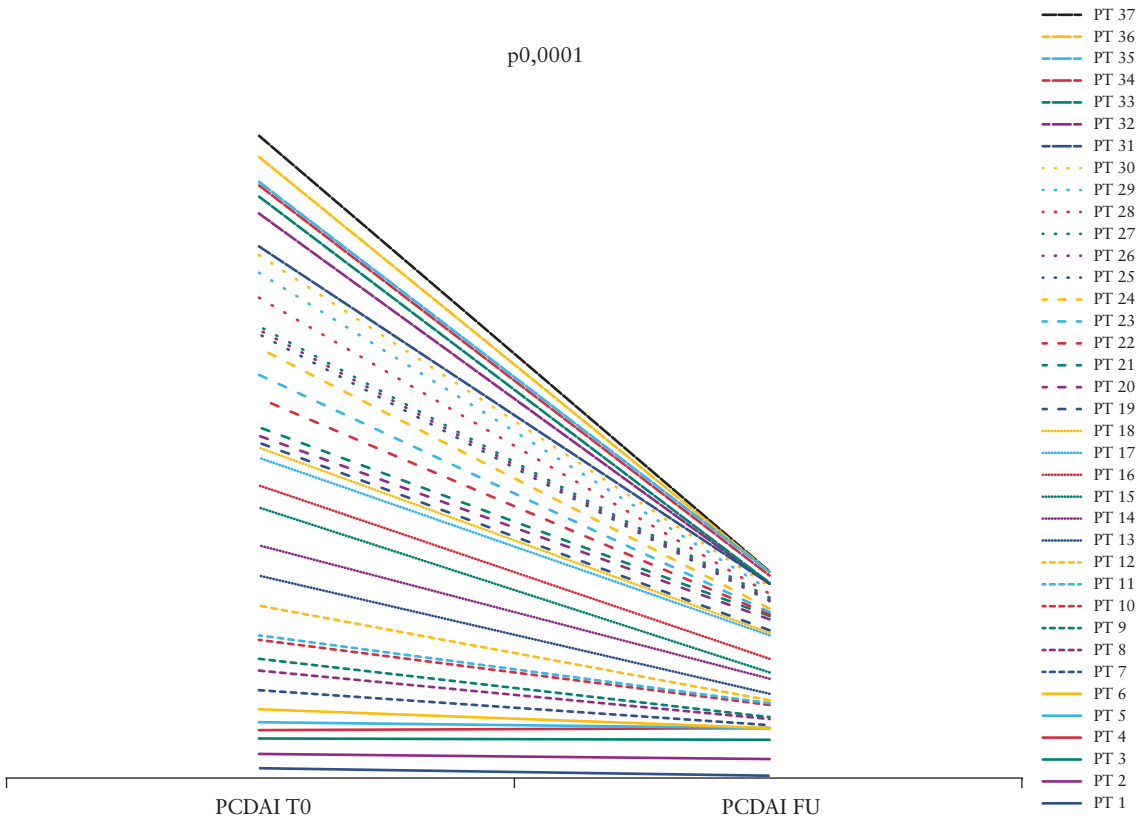

Figure 1. Pediatric Crohn's Disease Activity Index (PCDAI) values for each patient at baseline and at follow-up (FU).

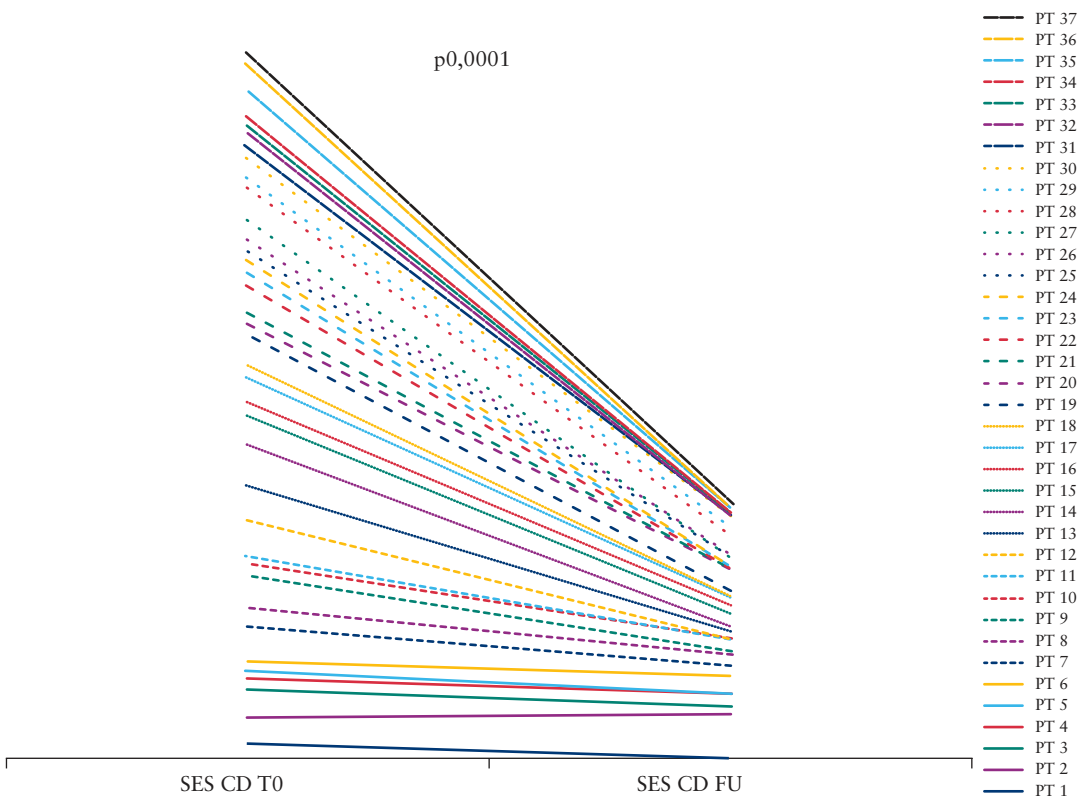

Figure 2. Simple Endoscopic Score for Crohn's Disease (SES-CD) values for each patient at baseline and at follow-up (FU).

Table 2. Multivariate analysis evaluating the association between patients' baseline characteristics and outcome at follow-up endoscopy.

	Complete mucosal healing		Partial + complete mucosal healing	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
SES-CD at T0	0.92 (0.83–1.01)		1.06 (0.96–1.17)	1.27 (1.02–1.57)
Immunomodulators	1.16 (0.29–4.59)		4.75 (1.06–21.36)	15.0 (1.66–135.7)
ESR at T0	0.95 (0.92–0.98)	0.91 (0.86–0.97)	0.99 (0.96–1.01)	0.94 (0.90–0.99)
Age at diagnosis (months)	1.011 (0.994–1.029)	1.05 (1.01–1.08)	1.012 (0.99–1.033)	
Age of starting therapy (months)	1.013 (0.995–1.031)		1.01 (0.99–1.03)	
Therapy				
Adalimumab (reference)	1		1	
Infliximab	0.74 (0.19–3.22)		1.29 (0.29–5.67)	
Timing of therapy				
Step up (reference)	1		1	
Top down	1.73 (0.45–6.63)		3.64 (0.78–16.93)	
PCR at T0	0.96 (0.75–1.21)		0.94 (0.74–1.20)	
Sex				
Male (reference)	1		1	
Female	0.52 (0.12–2.16)		1.09 (0.25–4.71)	

SES-CD, Simple Endoscopic Score for Crohn's Disease; ESR, erythrocyte sedimentation rate; PCR, polymerase chain reaction.

(p = 0.91 and p = 0.42). The same results were found in patients treated with adalimumab (p = 0.735).

If considering the combination of complete plus partial MH as the outcome, there was a statistical difference in favour of early therapy introduction ($p < 0.05$) only in the group of patients treated with infliximab.

The rates of complete, partial and no MH in the early disease group were 45, 39 and 16%, respectively, and those in the late disease group were 32, 26 and 42%, respectively.

Regarding the rates of remission and response according to PCDAI values at follow-up, we found no differences between the two groups of patients, with rates of remission and response of 70.6% and 12% in the early group, and 70% and 10% in the late group, respectively.

3.4. Differences between immunomodulators plus infliximab combination and infliximab monotherapy

We further evaluated the influence of combination therapy with anti-TNFα plus azathioprine or methotrexate versus anti-TNFα monotherapy on the achievement of MH and clinical improvement. In both groups SES-CD values decreased significantly at follow-up, but the reduction was more significant in the combination therapy group (p = 0.0002 vs p = 0.033). The two groups did not differ in the achievement of complete MH (p = 0.835). However, when considering complete plus partial MH as the outcome, the combination therapy group had significantly better results (p = 0.035) (Figure 3).

The rates of complete MH were similar in the two groups, combination therapy vs monotherapy, (37.5 vs 33%) but the rate of no MH was considerably higher in the monotherapy one (18.75 vs 46.6%). The rates of clinical remission according to PCDAI values at follow-up did not differ statistically between the two groups (combination, 74%; monotherapy, 64%). The rate of non-response at follow up was 13% in the combination group and 21% in the monotherapy one (not significant).

3.5. Follow-up after second endoscopy

To evaluate the influence of the achievement of MH on subsequent follow-up we analysed the clinical outcome 1 and 2 years after the second endoscopy.

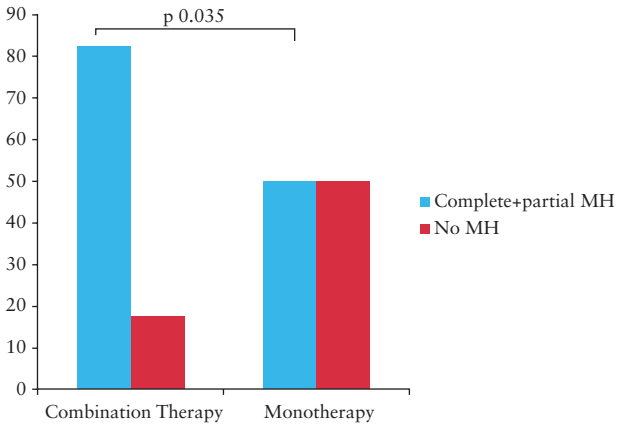

Figure 3. Rates of complete mucosal healing (MH) + partial MH and no MH according to combination therapy with anti-tumour necrosis factor α (TNFα) and immunomodulators vs anti-TNFα monotherapy.

At the first evaluation, 12 months after the follow-up endoscopy, 26 patients (70.3%) still on anti-TNFα maintenance therapy were in clinical remission; 4 patients (10.8%), although on maintenance therapy, were not in clinical remission and the remaining 7 (18.9%) had interrupted therapy for either surgery (4 patients) or loss of response (3 patients).

All the patients that had achieved complete MH and 75% of those that had achieved partial MH were in clinical remission at this assessment. Evaluating the need for corticosteroids in this further follow-up, 2 patients that had obtained complete MH and 4 of the patients that had obtained partial MH needed a short course of corticosteroids.

Kaplan–Meier survival curves evaluating the risk of disease relapse 2 years after therapy introduction demonstrated no statistical difference according to treatment (adalimumab vs infliximab) (p = 0.089) (Figure 4). The same evaluation could not be performed because of the risk of surgery, since no patient on adalimumab underwent surgical treatment.

All patients underwent a second clinical assessment 24 months after the follow-up endoscopy: 59.5% of all patients were still in

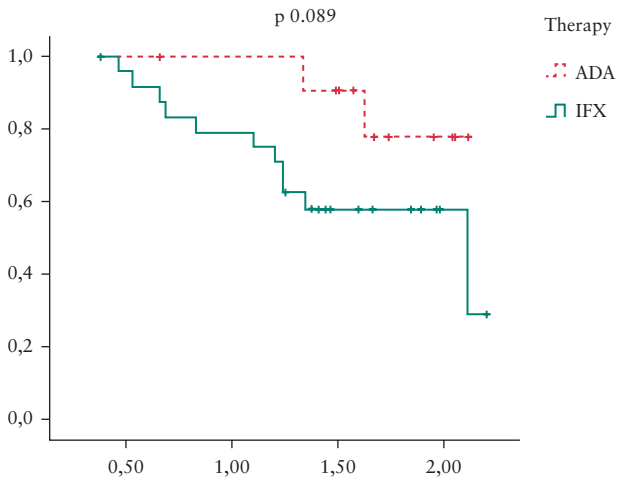

Figure 4. Kaplan–Meier curve evaluating the risk of disease relapse according to therapy at a further follow-up 1 year after the second endoscopic evaluation.

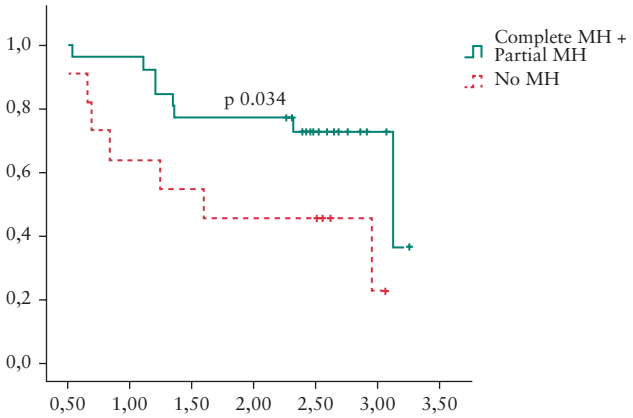

Figure 5. Kaplan–Meier curve evaluating relapse risk according to the achievement of mucosal healing (MH) at a further follow-up 2 years after the second endoscopic evaluation.

clinical remission on anti-TNFα maintenance therapy; 8% of all patients, although on maintenance therapy, were not in clinical remission and the remaining 12 (32.5%) had discontinued treatment because of surgery (3 patients), loss of response (2 patients), switch to the other anti-TNFα (6 patients) or sustained remission (1 patient).

Of the patients that had achieved complete MH, 78.6% were still in clinical remission and 2 required a brief steroid course during this further follow-up. Regarding patients that had achieved partial MH, 67% were in clinical remission and another 2 required a course of steroids at this further evaluation.

Kaplan–Meier curves evaluating the risk of relapse during the 2 years after the follow-up endoscopy according to the achievement of MH showed no statistical difference when considering the three groups (complete MH, partial MH and no MH) separately; the same evaluation when the patients were divided into two groups (complete plus partial MH together compared with no MH) showed a statistical difference in the risk of relapse in favour of the first group (p = 0.034) (Figure 5).

4. Discussion

Until a few years ago, clinical response and remission were the major goals in therapeutic trials of IBD patients. Since the advent of disease-modifying drugs, such as biological agents, it became possible that MH could be a reliable outcome in the management of IBD patients.

Despite the fact that corticosteroids can induce MH to a certain degree in UC patients, they appear unable to promote effective healing of ulceration in CD patients. Such an outcome has been related to sustained long-term remission and surgery-free survival, thus changing the disease course of IBD.[7,8] Immunomodulators, such as thiopurines, and the polymeric diets given through continuous enteral nutrition, have been previously reported to achieve MH, albeit to a lower degree and less rapidly than biologics.[8–10]

Although biological agents have proven effective in obtaining rapid clinical remission also in paediatric patients,[20–28] scarce evidence is available of the effect on MH in paediatric cohorts.[17,18] Borrelli et al.[17] evaluated endoscopic activity after an induction regimen of infliximab in a paediatric cohort of 18 patients affected by CD; in this cohort both CDEIS and the histological score were significantly reduced at the follow-up endoscopy. A more recent study that aimed to assess clinical efficacy and the impact of infliximab induction therapy on MH in a paediatric cohort showed that complete MH was achieved in 22.7% of patients at week 10 after therapy initiation.[18]

Our study aimed to assess such a crucial outcome in a cohort of paediatric CD subjects who were naive to biological treatments, and revealed that a high proportion of our patients achieved substantial MH. Since previous studies in paediatric CD did not assess clear rates of MH as an endpoint but only demonstrated a significant decrease in endoscopic scores,[17,18] our data can be compared with the rates available in adult studies, although biased by the populations' heterogeneity and different endoscopic timing.

The rate of complete MH in our population (38% overall) was lower than previously reported in adults on infliximab treatment; in the latter, rates of complete MH at follow-up ranged from 44–45.4 to 68%.[7,11,12] These differences could be related to the more aggressive phenotype and the more extensive disease usually seen in paediatric patients.[2–4] Indeed, the extrapolation of the rate of complete MH in a recently published paediatric study, showing a rate of complete MH at follow-up endoscopy of 22.7%, was similar to our findings.[18] On the other hand, such a difference appears less remarkable when looking at the EXTEND trial, the only available trial evaluating MH in adalimumab-treated adult patients that shows a complete MH rate of 27% at week 12.[13] Undeniably, the different endoscopic timing between the EXTEND trial and our study does not allow a direct comparison but, bearing in mind the proportion of late responders in adalimumab patients, it is conceivable that our higher MH rate is a result of this trend.

Lately, the use of concomitant immunomodulator treatment has been controversial, mainly for safety reasons; however, a synergic effect on therapeutic efficacy has been suggested.[29] In our population, no statistical difference in obtaining complete MH between monotherapy and combination therapy was found. This result seems to be in agreement with previous adult and paediatric data reporting no significant difference in achieving clinical remission.[11,12,25] Interestingly, when complete MH plus partial MH were considered as the outcome, the difference between the two groups was significant. Indeed, complete as well as partial healing induced by long-term maintenance treatment has been associated with an improved long-term outcome of CD, especially with a lower need for major abdominal surgeries.[11] Therefore, the degree of MH that is required to change the course of CD is still debated.[30]

The concept of the 'window of opportunity' for a better treatment outcome in early CD, when the disease is still in a predominantly

inflammatory situation and disease complications have not yet appeared, has recently been adopted from the rheumatology literature.[31] Yet, in contrast with previous data,[32–34] in our cohort no statistical difference in achieving MH according to disease duration was found. A possible explanation is that in our cohort the median disease duration prior to the introduction of biological therapy was only 13 months, so most of our patients could be classified as having relatively early disease, with a predominantly inflammatory pattern.

Long-term evaluations of adult patients with IBD have shown a decreased rate of surgeries and hospitalization in those achieving MH, thus suggesting that the accomplishment of such a treatment goal could predict a better outcome, possibly altering the natural course of CD.[11,12] Indeed, in our cohort a further clinical follow-up of 2 years from the second endoscopic evaluation showed that a high percentage of patients (100% at 1 year and 79% at 2 years) with complete MH and most of those with partial MH (75 and 67% at 1 and 2 years, respectively) were still in clinical remission on maintenance therapy. Most importantly, during this further follow-up period, only four of the patients with complete MH needed a corticosteroid course and none underwent surgery. Moreover, no statistical difference in the risk of relapse between the two therapies has been found. Evaluating the relapse risk, dividing patients according to the achievement of MH, complete plus partial MH vs no MH, showed that the first group had a significantly lower risk of relapse.

We are aware that our data should be interpreted with the following limitations: (1) the small size of the patient cohort; and (2) the follow-up period is not long enough, especially for those that discontinue treatment because of clinical and endoscopic remission, to allow better evaluation of the impact of MH on long-term outcomes.

In conclusion, our results have shown that biological therapy with infliximab and adalimumab is effective in achieving MH, at least in markedly improving mucosal lesions in paediatric CD patients. In our cohort, disease duration or combination therapy did not influence significantly the patients' outcome, although the combination of biologics and immunomodulators was more efficacious than biological monotherapy in improving mucosal lesions.

At a further 2 years of clinical follow-up, the improvement of mucosal lesions at the second endoscopy appears predictive of a better outcome.

Finally, since CD is known to be a transmural disease, with complications such as strictures, abscesses and/or fistulas, transmural healing should be considered in the near future for potential inclusion in the definition of deep remission for CD.

Funding

This work was performed with no funding.

Conflict of Interest

All authors have no conflict of interest to declare.

Acknowledgments

Each author made a substantial contribution to the conception and design of the study, the acquisition of data or analysis and interpretation of data, to drafting the article or revising it critically for important intellectual content, and has approved the final version to be submitted.

The specific contributions of each author were as follows: FN and FV designed the study; FC, MA, FN and FV performed clinical follow-up; SO performed the endoscopic evaluations; SB and SO collected the data; GL did the statistical analyses; FN wrote the first draft of the manuscript; SC and FV critically reviewed the draft and approved the version to be published. The manuscript has been approved by all the authors.

Presented as poster presentation at ESPGHAN 2014, Jerusalem 9–12, June 2014.

References

1. Benchimol EI, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis* 2011;**17**:423–39.
2. Vernier-Massouille G, Balde M, Salleron J, *et al.* Natural history of pediatric Crohn's disease: a population-based cohort study. *Gastroenterology* 2008;**135**:1106–13.
3. Pigneur B, Seksik P, Viola S, *et al.* Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. *Inflamm Bowel Dis* 2010;**16**:953–61.
4. Sauer CG, Kugathasan S. Pediatric inflammatory bowel disease: highlighting pediatric differences in IBD. *Med Clin North Am* 2010;**94**:35–52.
5. Cosnes J, Cattan S, Blain A, *et al.* Long-term evolution of disease behavior of Crohn's disease. *IBD* 2002;**8**:244–250
6. Romberg-Camps MJ, Dagnelie PC, *et al.* Influence of phenotype at diagnosis and of other potential prognostic factors on the course of inflammatory bowel disease. *Am J Gastroenterol* 2009;**104**:371–83.
7. Baert F, Moortgat L, Van Assche G, *et al.,* Belgian Inflammatory Bowel Disease Research Group; North-Holland Gut Club. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. *Gastroenterology* 2010;**138**:463–8.
8. Pineton de Chambrun G, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. *Nat Rev Gastroenterol Hepatol* 2010;**7**:15–29.
9. Mantzaris GJ, Christidou A, Sfakianakis M, *et al.* Azathioprine is superior to budesonide in achieving and maintaining mucosal healing and histologic remission in steroid-dependent Crohn's disease. *Inflamm Bowel Dis* 2009;**15**:375–82.
10. Peyrin-Biroulet L, Reinisch W, Colombel JF, *et al.* Clinical disease activity, C-reactive protein normalisation and mucosal healing in Crohn's disease in the SONIC trial. *Gut* 2014;**63**:88–95.
11. Schnitzler F, Fidder H, Ferrante M, *et al.* Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. *Inflamm Bowel Dis* 2009;**15**:1295–301.
12. Rutgeerts P, Diamond RH, Bala M, *et al.* Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. *Gastrointest Endosc* 2006;**63**:433–42.
13. Rutgeerts P, Van Assche G, Sandborn WJ, *et al.,* EXTEND Investigators. Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: data from the EXTEND trial. *Gastroenterology* 2012;**142**:1102–1111.
14. Rismo R, Olsen T, Ciu G, *et al.* The effect of adalimumab for induction of endoscopic healing and normalization of mucosal cytokine gene expression in Crohn's disease. *Scand J Gastroenterol* 2012; **47**: 1200–10.
15. Zallot C, Peyrin-Biroulet L. Deep remission in inflammatory bowel disease: looking beyond symptoms. *Curr Gastroenterol Rep* 2013;**15**:315.
16. Molander P, Sipponen T, Kemppainen H, *et al.* Achievement of deep remission during scheduled maintenance therapy with TNFα-blocking agents in IBD. *J Crohns Colitis* 2013 1;**7**:730–5.
17. Borrelli O, Bascietto C, Viola F, *et al.* Infliximab heals intestinal inflammatory lesions and restores growth in children with Crohn's disease. *Dig Liver Dis* 2004;**36**:342–7.
18. Kierkus J, Dadalski M, Szymanska E, *et al.* The impact of infliximab induction therapy on mucosal healing and clinical remission in Polish pediatric patients with moderate-to-severe Crohn's disease. *Eur J Gastroenterol Hepatol* 2012;**24**:495–500.
19. Daperno M, D'Haens G, Van Assche G, *et al.* Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004;**60**:505–512.

20. Hyams J, Crandall W, Kugathasan S, *et al.* REACH Study Group. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007;**132**:863–73.
21. De Bie CI, Hummel TZ, Kindermann A, *et al*. The duration of effect of infliximab maintenance treatment in pediatric Crohn's disease is limited. *Aliment Pharmacol Ther* 2011; **33**:243–250.
22. Hyams JS, Lerer T, Griffiths A, *et al*. Long-term outcome of maintenance infliximab therapy in children with Crohn's disease. *Inflamm Bowel Dis* 2009; **15**:816–822.
23. Crombè V, Salleron J, Savaye G, *et al.* Long-term outcome of treatment with infliximab in pediatric-onset Crohn's disease: a population-based study. *Inflamm Bowel Dis* 2011;**17**:2144–52.
24. Assa A, Hartman C, Weiss B, *et al.* Long-term outcome of tumor necrosis factor alpha antagonist's treatment in pediatric Crohn's disease. *J Crohns Colitis* 2013;**7**:369–76.
25. Nuti F, Viola F, Civitelli F, *et al.* Biological therapy in a pediatric Crohn disease population at a referral center. *J Pediatr Gastroenterol Nutr* 2014;**58**:582–7.
26. Noe JD, Pfefferkorn M. Short-term response to adalimumab in childhood inflammatory bowel disease. *Inflamm Bowel Dis* 2008;**14**:1683–7.
27. Viola F, Civitelli F, Di Nardo G, *et al.* Efficacy of adalimumab in moderate-to-severe pediatric Crohn's disease. *Am J Gastroenterol* 2009;**104**:2566–71.
28. Rosh JR, Lerer T, Markowitz J, *et al*. Retrospective evaluation of the safety and effect of adalimumab therapy (RESEAT) in pediatric Crohn's disease. *Am J Gastroenterol* 2009;**104**:3042–9.
29. Colombel JF, Sandborn WJ, Reinisch W, *et al*., SONIC Study Group. Infliximab, azathioprine, or combination therapy for Crohn's disease. SONIC Study Group. *N Engl J Med* 2010;15;**362**:1383–95.
30. Peyrin-Biroulet L, Ferrante M, *et al.,* Scientific Committee of the European Crohn's and Colitis Organization. Results from the 2nd Scientific Workshop of the ECCO. I: impact of mucosal healing on the course of inflammatory bowel disease. *J Crohns Colitis* 2011;**5**:477–83.
31. Danese S, Fiorino G, Fernandes C, Peyrin-Biroulet L. Catching the therapeutic window of opportunity in early Crohn's disease. *Curr Drug Targets* 2014;**15**;1056–1063.
32. Lionetti P, Bronzini F, Salvestrini C, *et al.* Response to infliximab is related to disease duration in paediatric Crohn's disease. *Aliment Pharmacol Ther* 2003;**18**:425–31.
33. Kugathasan S, Werlin SL, Martinez A, Rivera MT, Heikenen JB, Binion DG. Prolonged duration of response to infliximab in early but not late pediatric Crohn's disease. *Am J Gastroenterol* 2000;**95**:3189–94.
34. Lee JS, Lee JH, Lee JH, *et al*. Efficacy of early treatment with infliximab in pediatric Crohn's disease. *World J Gastroenterol* 2010;**16**:1776–81.