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Mucosal Healing in Paediatric Patients with Moderate-to-Severe Luminal Crohn's Disease Under Combined Immunosuppression: Escalation versus Early Treatment

Ben Kang[a], So Yoon Choi[a], Hye Seung Kim[b], Kyunga Kim[b], Yoo Min Lee[c], Yon Ho Choe[a]

[a]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea [b]Biostatistics and Clinical Epidemiology Center, Research Institute for Future Medicine, Samsung Medical Center, Seoul, Korea [c]Department of Pediatrics, Kyung Hee University Graduate School of Medicine, Seoul, Korea

Corresponding author: Yon Ho Choe, MD, PhD, Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135–710, Korea. Tel: 82-2-3410-3527; fax: 82-2-3410-0043; email: i101016@skku.edu

Abstract

Background and Aims: We aimed to compare the efficacy of combined immunosuppression in terms of mucosal healing in paediatric patients with moderate-to-severe luminal Crohn's disease receiving infliximab according to either an 'escalated combined immunosuppression' or an 'early combined immunosuppression' strategy.

Methods: In this prospective observational study, the efficacy of combined immunosuppression was evaluated in terms of mucosal healing at weeks 14 and 54 from baseline infliximab infusion. Comparison was performed between the escalated combined immunosuppression group [group A] and the early combined immunosuppression group [group B]. Factors associated with mucosal healing at weeks 14 and 54 from baseline infliximab infusion were also investigated.

Results: Seventy-six patients initiated infliximab with concomitant azathioprine [group A = 28; group B = 48]. Comparison of baseline characteristics revealed a significantly longer duration from initial diagnosis to infliximab infusion in group A [median 8.1 vs. 0.7 months; $p < 0.001$]. Mucosal healing was achieved in 32% of patients in group A and 51% in group B at week 14 [$p = 0.121$], and in 42% in group A and 74% in group B at week 54 [$p = 0.007$]. Group B was also positively associated with mucosal healing at week 54 on multivariate logistic regression [odds ratio = 6.216, 95% confidence interval = 1.782–21.686, $p = 0.004$].

Conclusions: Mucosal healing during combined immunosuppression is more effectively achieved by treatment with an early combined immunosuppression strategy without corticosteroid induction administered within 1 month rather than escalating to receive combination therapy later during the course. The therapeutic window of opportunity in early Crohn's disease may be shorter than generally thought, especially in children.

Key Words: mucosal healing; paediatric Crohn's disease; combined immunosuppression

1. Introduction

Crohn's disease [CD] is a chronic progressive inflammatory bowel disease [IBD] that can affect the entire gastrointestinal tract, often leading to significant complications requiring surgery and consequently to impaired quality of life.[1] Although the traditional goals of treatment in CD have been aimed at controlling symptoms by inducing and maintaining clinical remission, optimizing quality of life, and reducing structural complications related to disease progression, mucosal healing [MH] has recently received increasing attention as a major potential treatment goal in CD.[2] The importance of MH derives from previous large-scale studies with anti-tumour necrosis factor [TNF] agents that have shown that the achievement of MH is associated with reduced hospitalization and surgery rates and sustained corticosteroid [CS]-free remission, which may reflect modification of the natural history of CD.[3,4,5,6] However, its integration into clinical trials and real-life clinical practice is limited in the paediatric population because of the invasiveness of ileocolonoscopy and the consequent limitation for repetitive performance. Therefore, current literature regarding MH in the paediatric population is scarce, especially among patients receiving biologics.[7,8,9]

Among the currently available drugs, biologics demonstrate the most profound impact on MH in CD, which is further augmented by their earlier introduction in the disease course.[6,10] In our previous study of patients who were treated by combined immunosuppression with infliximab [IFX] and azathioprine [AZA] according to either a 'step-up' or a 'top-down' approach from diagnosis, deep remission rates at 1 year from baseline IFX were significantly higher in the 'top-down' group.[11] Our main interest in that study was to investigate the efficacy of combined immunosuppression between the two different therapeutic approaches in terms of relapse-free rates 3 years from the start of IFX. In the present study we aimed to compare the efficacy of combined immunosuppression in terms of MH at the time points of post-induction and 1 year maintenance from baseline IFX between patients who received IFX according to an early combined immunosuppression strategy, which was performed within 1 month from diagnosis without CS induction, and those who were escalated to initiate IFX according to a conventional 'step-up' strategy initiated with CS induction. We also aimed to analyse factors associated with MH at these time points to investigate whether an early combined immunosuppression strategy without CS induction was associated with MH during IFX treatment.

2. Materials and methods

2.1. Patients and study design

This study was a prospective observational study conducted at the Department of Pediatrics, Samsung Medical Center, between January 2011 and December 2014. Subjects enrolled in this study were paediatric patients diagnosed with moderate-to-severe luminal CD of non-penetrating, non-stricturing behaviour. Patients of indeterminate-type IBD, mild disease activity at diagnosis, penetrating or stricturing disease behaviour, previous history of bowel surgery, IFX indicated for the treatment of refractory perianal fistulas, and age at IFX baseline of 18 years or over were excluded. CD was diagnosed in accordance with the revised Porto criteria of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition [ESPGHAN].[12] Moderate-to-severe CD at diagnosis was defined as a score of 30 points or greater according to the Pediatric Crohn's Disease Activity Index [PCDAI].[13] Disease classification and behaviour was based on the Paris classification.[14] This study was approved by the Institutional Review Board of Samsung Medical Center and was conducted in accordance with the *Declaration of Helsinki*.

Enrolled subjects and their guardians were allowed to choose their initial treatment between either a conventional step-up strategy initiated by CS induction or an early combined immunosuppression strategy without CS induction after thorough explanation of the pros and cons of each treatment strategy. The investigator was not involved in decision-making of which treatment strategy would be initiated, and written consent was obtained from the subjects and their guardians. Subjects who chose to escalate treatment to IFX when induction based on CS failed or when disease relapsed during maintenance with AZA after initially achieving clinical remission with CS were allocated to the step-up group. Those among the step-up group who were eventually escalated to receive IFX were included in the 'escalated combined immunosuppression group' [group A]. Subjects who chose early combination treatment with IFX and AZA within 1 month from diagnosis without CS induction were allocated to the 'early combined immunosuppression' group [group B].

Treatment in the step-up group was started with oral CS at a dose of 1 mg/kg/day [maximum 60 mg/kg/day] and was tapered over 8 weeks. Oral AZA and mesalazine was also started with oral CS. When treatment response was refractory to or dependent on CS, IFX was started with no changes in the treatment with AZA and mesalazine. For patients who were capable of maintaining clinical remission after cessation of CS, oral AZA and mesalazine were continued; however, IFX was initiated if the disease later relapsed during the course of treatment. Clinical relapse was defined as a PCDAI greater than 10 points after achievement of clinical remission.[13] For patients in group B, treatment was initiated within 1 month from diagnosis with intravenous IFX together with oral AZA and mesalazine. Mesalazine was given concomitantly with AZA to improve treatment response and reduce thiopurine-related toxicity by increasing the levels of 6-thioguanine nucleotides [6-TGN].[15] Partial exclusive nutrition [PEN] was given both during CS induction in those who were allocated to the step-up group, and also during induction with IFX in groups A and B. Formulas used for enteral nutrition were extensively hydrolysed formulas for CD patients, namely Elemental 028® Extra [Nutricia Clinical Care, Trowbridge, UK] or Monowell® [Korea Medical Food Co. Ltd, Seoul, Korea].

In both groups IFX was administered according to the scheduled induction regimen of 5 mg/kg at weeks 0, 2, and 6, and no dose adjustments were allowed during this period. Disease activity was assessed at week 14 from IFX initiation based on PCDAI and patients who showed primary non-response to IFX were dropped from the analysis. Scheduled IFX was repeated every 8 weeks starting from week 14. Dose intensification of IFX by interval shortening or dosage increment was allowed when a clinical relapse was observed at the consecutive follow-up, as is currently practised worldwide and also recommended in the treatment to overcome secondary loss of response. AZA was given at doses of 0.5–1 mg/kg/day and was later modified when required.[15,16,17] The requirement for dose modification was based on relevant adverse events, laboratory examinations, thiopurinemethyltransferase [TPMT] genotype results, and thiopurine metabolite levels of 6-TGN and 6-methylmercaptopurine [6-MMP]. Thiopurine metabolite levels were checked every 1–3 months after 3 months of treatment with AZA. Doses were targeted such that 6-TGN levels ranged from 235 to 450 pmol/8×10^8 red blood cells [RBCs] and 6-MMP level was <5 700 pmol/8×10^8 RBCs.[18] Mesalazine was given at a dose of 50 mg/kg/day. Both groups received partial enteral nutrition during the IFX induction phase.

The main focus of investigation in this study was to compare the efficacy of combination treatment in terms of MH between two study groups that had received IFX, rather than the effectiveness in terms of MH between 'step-up' and 'early combined immunosuppression' treatment strategies starting from diagnosis. Thus, baseline ileocolonoscopy was scheduled prior to baseline IFX and follow-up ileocolonoscopy was scheduled at weeks 14 and 54 from baseline IFX. Only those subjects who underwent ileocolonoscopy at these time points were included in the analysis. Subjects who had not started IFX or those who had stopped IFX despite dose intensification during the study period consequently lacked endoscopic evaluation at a time point and were excluded from analysis for that particular time point. Ileocolonoscopies were planned under sedation with intravenous midazolam and pethidine.

Baseline clinicodemographic data including sex, birth date, disease classification, growth indicators, prior history of surgery of the gastrointestinal tract or anal fistulas, and family history of IBD were recorded at diagnosis. Physical examination, PCDAI scores, growth indicators, and laboratory examinations including complete blood cell counts with differential counts, chemistry profiles, erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP] were assessed prior to every infusion after IFX commencement. Trough serum levels of antibody to infliximab [ATI] were measured at week 54 from baseline IFX using an enzyme-linked immunosorbent assay [ELISA] kit [Matriks Biotek Laboratories, Ankara, Turkey].[19] Simple Endoscopic Score for Crohn's Disease [SES-CD] was assessed at each ileocolonoscopic examination to evaluate the degree of mucosal involvement.[20] Z-scores for weight-for-age, height-for-age, and body mass index [BMI]-for-age were calculated using the growth charts of the Centres for Disease Control and Prevention of Korea.

2.2. Outcomes and definition

The primary outcome of this study was MH at weeks 14 and 54 from baseline IFX. MH was defined as an SES-CD score of 0, which corresponds to complete healing of the mucosa to a normal status without any ulcers or other mucosal lesions such as erosion and inflammation. Secondary outcomes included clinical remission and laboratory remission at weeks 14 and 54 from baseline IFX. Tertiary outcomes included Z-scores at week 54 from baseline IFX and delta Z-scores of weight-for-age, height-for-age, and BMI-for-age between baseline IFX and week 54 from baseline IFX. Clinical remission was defined as a PCDAI of 10 points or less and laboratory remission was defined as CRP <0.5 mg/dl. Adverse events during the study period were also investigated.

2.3. Analysis of factors associated with mucosal healing

To investigate factors associated with MH at weeks 14 and 54 from baseline IFX, multivariate logistic regression analyses were performed with a stepwise selection procedure. Variables included for analysis at week 14 from baseline IFX were sex, diagnosis age, group, disease location, perianal fistulas, deep ulcers, PCDAI scores, SES-CD scores, albumin, ESR, and CRP levels at baseline IFX. For the analysis at week 54 from baseline IFX, clinical remission status, laboratory remission status, and MH status at week 14 from baseline IFX were added to the above variables.

2.4. Statistical analysis

For comparison of variables between two groups, two-sample t-test, Wilcoxon rank sum test, chi-square test, and Fisher's exact test were used as appropriate, and the p value for statistical significance was defined as $p < 0.05$. Power analysis was conducted for comparison of MH rates at weeks 14 and 54, and showed that the significance of a group difference in MH rate can be declared at a power of 80% or higher with a significance level of 5% when the difference was at least 33%. Logistic regression analysis based on last observation carried forward [LOCF] was used for univariate and multivariate analyses with stepwise selection to investigate factors associated with MH at weeks 14 and 54 from baseline IFX. Univariate logistic regression analysis was first conducted to investigate crucial odds ratio [OR] for each of the possible risk factors. Then, we further used multivariate logistic regression with a stepwise selection procedure to investigate adjusted ORs for significant risk factors after adjusting possible confounding among them. Because the stepwise procedure was applied, only factors showing multivariate-level significance with $p < 0.05$ were included in the multivariate logistic model. Results were expressed as OR with 95% confidence interval [CI]. Statistical analysis was carried out using SAS version 9.4 [SAS Institute, Cary, NC, USA].

3. Results

3.1. Baseline characteristics and clinical course

A total of 78 patients were initially enrolled in this observational study. Thirty patients were allocated to the step-up group. Two patients in the step-up group had maintained clinical remission with AZA and mesalazine after induction with CS for 15.3 and 26.8 months, respectively; therefore, 28 patients were finally included in the 'escalated combined immunosuppression group' [group A]. The other 48 patients were allocated to the 'early combined immunosuppression group' [group B], and started IFX with AZA and mesalazine within 1 month of diagnosis. The mean ± standard deviation [SD] for 6-TGN levels of the 28 patients in group A before IFX initiation was 306.5 ± 136.1 pmol/8 × 10^8 RBCs. Nine patients [32%] showed 6-TGN levels lower than 235 pmol/8 × 10^8 RBCs. Seven patients [25%] had taken less than 50% of their prescribed oral medication.

There was no significant difference between the two groups except for the duration from initial diagnosis to IFX infusion [median 8.1 months vs. 0.7 months; $p < 0.001$; Table 1]. During the induction phase, three patients in group A and one patient in group B were early IFX terminators due to primary non-response. Evaluation of clinical, laboratory, and endoscopic outcomes at week 14 from baseline IFX was performed in 25 patients in group A and 47 patients in group B. These subjects entered the maintenance phase. One patient in group A discontinued IFX before week 54 from baseline IFX due to secondary loss of response despite dose intensification of IFX and was therefore was switched to adalimumab [ADA]. Evaluation of clinical, laboratory, and endoscopic outcomes at week 54 from baseline IFX was performed in 24 patients in group A and 47 patients in group B [Figure 1]. Between weeks 14 and 54 from baseline IFX, dose intensification of IFX was performed in five of 24 patients [21%] in group A, and in two of 47 patients [4%] in group B [$p = 0.04$]. The median duration from baseline IFX to dose intensification was 48 weeks [range: 32–48 weeks]. Among the patients who had undergone evaluation of ATI at week 54, ATI was detected in three of 21 patients in group A and two of 44 patients in group B [$p = 0.318$]. All of the patients in whom ATIs were detected at week 54 from baseline IFX had received dose intensification between weeks 14 and 54. None of the patients

Table 1. Characteristics at baseline infliximab infusion.

	Group A [*n* = 28]	Group B [*n* = 48]	*p*
Male sex, *n* [%]	19 [67%]	30 [63%]	0.638
Age at diagnosis [years], median [range]	14.2 [8.8–17.0]	15.0 [8.5–17.8]	0.166
Age at IFX [years], median [range]	15.5 [10.4–17.7]	15.0 [8.6–17.9]	0.678
Duration from diagnosis to IFX infusion [months], median [range]	8.1 [1.9–33.2]	0.7 [0.1–0.9]	<0.001
Diagnosis age, *n* [%]			0.655
A1a	2 [7%]	3 [6%]	
A1b	24 [86%]	38 [79%]	
A2	2 [7%]	7 [15%]	
Lower GI tract location, *n* [%]			1.000
L1	0 [0%]	0 [0%]	
L2	2 [7%]	4 [8%]	
L3	26 [93%]	44 [92%]	
Upper GI tract location, *n* [%]			0.936
No involvement	10 [36%]	18 [37%]	
L4a	4 [14%]	8 [17%]	
L4b	10 [36%]	15 [31%]	
L4a+b	4 [14%]	7 [14%]	
Perianal fistulas, *n* [%]	17 [61%]	30 [63%]	0.877
1st degree history of IBD, *n* [%]	2 [7%]	4 [8%]	1.000
Weight Z-score, mean±SD	−0.80 ± 1.21	−0.82 ± 1.04	0.781
Height Z-score, mean±SD	−0.06 ± 1.05	−0.05 ± 1.03	0.962
BMI Z-score, mean±SD	−0.96 ± 1.04	−1.00 ± 1.09	0.833
Height Z-score ≤ −1.65, *n* [%]	3 [11%]	1 [2%]	0.139
Tanner stage I–II, *n* [%]	7 [25%]	12 [25%]	1.000
PCDAI, median [range]	35 [30–72.5]	35 [30–75]	0.723
WBC count [/μl], median [range]	7100 [3650–14390]	7300 [4240–13530]	0.401
Haematocrit [%], median [range]	35.4 [27.7–43.3]	34.7 [25.1–44.3]	0.921
Platelet count [×1000/μL], median [range]	358 [276–623]	408 [246–683]	0.16
Albumin [g/dl], median [range]	3.8 [2.8–4.4]	3.7 [2.3–4.5]	0.348
ESR [mm/h], median [range]	58 [23–120]	64 [21–120]	0.838
CRP [mg/dl], median [range]	1.24 [0.4–5.65]	1.19 [0.26–7.51]	0.73
CRP ≥ 0.5 mg/dl, *n* [%]	26 [93%]	45 [94%]	1.000
SES-CD, median [range]	17 [10–30]	18 [10–28]	0.694
Deep ulcers on ileocolonoscopy, *n* [%]	15 [54%]	29 [60%]	0.56

Group A, escalated combined immunosuppression group; Group B, early combined immunosuppression group; IFX, infliximab; A1a, 0–9 years; A1b, 10–17 years; A2, 17–18 years; GI, gastrointestinal; L1, distal 1/3 ileum ± limited coecal disease; L2, colonic disease; L3, ileocolonic disease; L4a, upper disease proximal to ligament of Treitz; L4b, upper disease distal to the ligament of Treitz and proximal to the distal 1/3 ileum; L4a+b, upper disease involvement in both L4a and L4b; IBD, inflammatory bowel disease; SD, standard deviation; BMI, body mass index; PCDAI, Pediatric Crohn's Disease Activity Index; WBC, white blood cell; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SES-CD, Simple Endoscopic Score for Crohn's Disease.

received CS during combination treatment with IFX and AZA. 6-TGN levels were checked at least once in 67 patients [range: 1–8 times]. The mean values of median 6-TGN levels of patients did not show a statistically significant difference between the two groups [326 ± 95 vs. 317 ± 97 pmol/8 × 10^8 RBCs, p = 0.719]. No patient in group A was dropped during the study period due to the aggravation of perianal fistulas requiring IFX, nor did any new perianal fistula develop in patients who did not demonstrate perianal fistulas at diagnosis.

3.2. Comparison of remission outcomes between groups

At week 14 from baseline IFX, MH rates were higher in group B than in group A, although a significant difference was not observed between the two groups [32% vs. 51%, p = 0.121]. In addition, other outcomes did not differ significantly between the two groups despite statistical significance between the two groups in CRP levels and SES-CD scores [Table 2].

At week 54 from baseline IFX, MH rates were significantly higher in group B than in group A [42% vs. 74%, p = 0.007], whereas there was no significant difference in clinical remission rates and laboratory remission rates between the two groups.

3.3. Analysis of factors associated with mucosal healing

Logistic regression analysis based on LOCF revealed that 'group B' was positively associated with MH at week 14 from baseline IFX [OR = 4.271, 95% CI = 1.249–14.605, p = 0.02], and 'upper gastrointestinal tract involvement' was negatively associated with MH at week 14 from baseline IFX [OR = 0.156, 95% CI = 0.041–0.589, p = 0.006]. Logistic regression analysis based on LOCF of factors associated with MH at week 54 from baseline IFX revealed that 'group B' and a 'healed mucosa status at week 14 from baseline IFX' were positively associated, and 'upper gastrointestinal tract involvement' and 'presence of perianal fistulas' were negatively associated with MH at week 54 [Table 3].

3.4. Growth indicators

Z-scores for weight-for-age, height-for-age, BMI-for-age, and weight-for-height at weeks 14 and 54 from baseline IFX did not

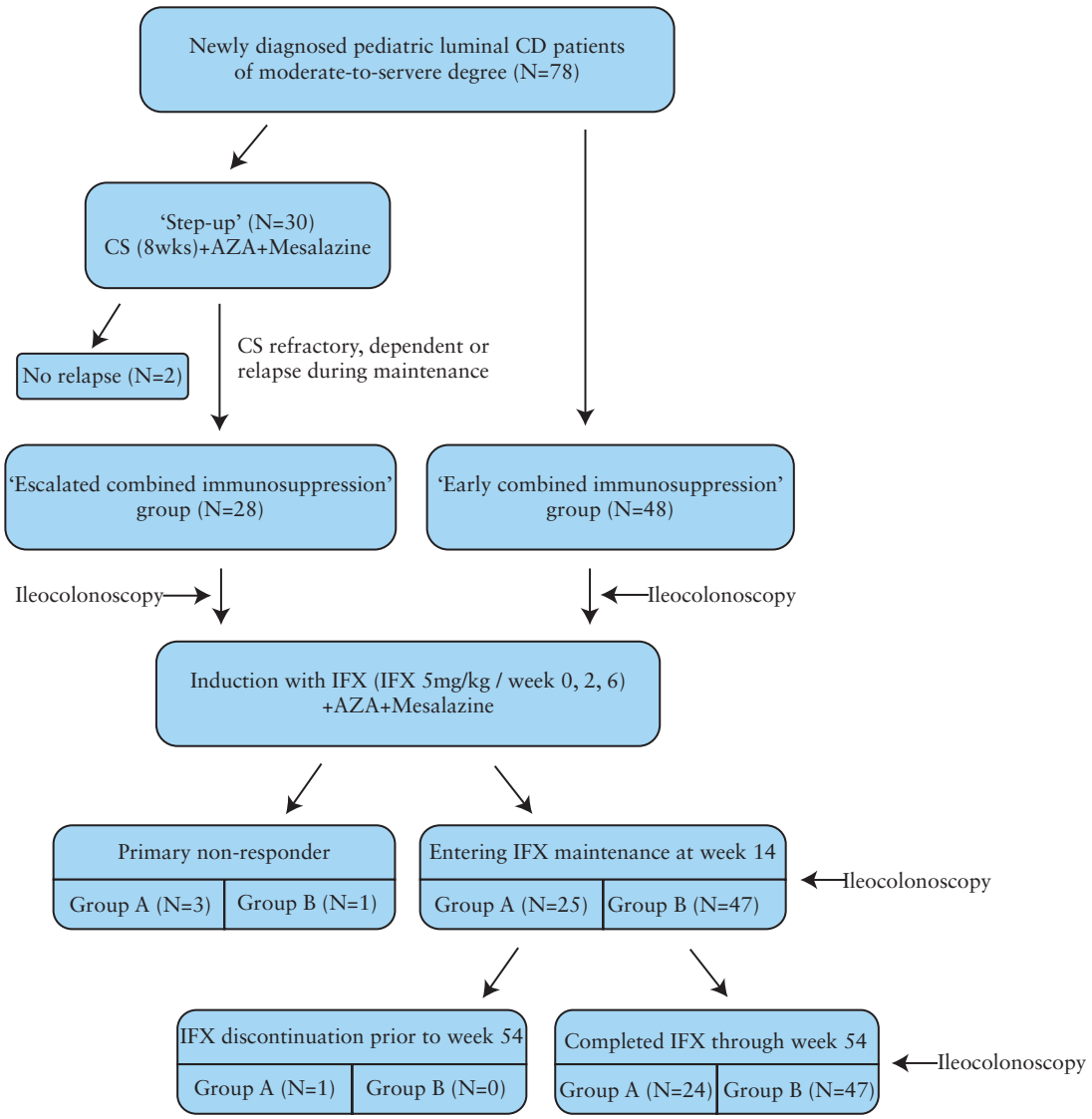

Figure 1. Schematic outline of patient inclusion, treatment, and ileocolonoscopic examination. CS, corticosteroid; IFX, infliximab; AZA, azathioprine.

Table 2. Clinical, laboratory, endoscopic, and growth outcomes at follow-up

	Week 14 from baseline IFX			Week 54 from baseline IFX		
	Group A [*n* = 25]	Group B [*n* = 47]	*p*	Group A [*n* = 24]	Group B [*n* = 47]	*p*
PCDAI, median [range]	5 [0–15]	5 [0–15]	0.517	3.75 [0–35]	0 [0–22.5]	0.049
Clinical remission, *n* [%]	20 [80%]	43 [91%]	0.26	19 [79%]	42 [89%]	0.289
CRP [mg/dl], median [range]	0.2 [0.03–1.96]	0.03 [0.03–1.44]	0.013	0.2 [0.03–4.04]	0.06 [0.03–1.28]	0.009
Laboratory remission, *n* [%]	18 [72%]	42 [89%]	0.095	16 [67%]	41 [87%]	0.058
SES-CD, median [range]	4 [0–21]	2 [0–18]	0.012	3 [0–22]	0 [0–19]	0.03
MH, *n* [%]	8 [32%]	24 [51%]	0.121	10 [42%]	35 [74%]	0.007
Weight Z-score, mean ± SD	−0.18 ± 0.75	−0.24 ± 0.93	0.781	−0.05 ± 0.82	0.00 ± 0.97	0.844
Height Z-score, mean ± SD	−0.03 ± 1.05	0.00 ± 1.02	0.91	0.03 ± 1.05	0.06 ± 1.02	0.927
BMI Z-score, mean ± SD	−0.20 ± 0.61	−0.30 ± 0.89	0.495	−0.08 ± 0.78	−0.04 ± 0.99	0.889

IFX, infliximab; Group A, escalated combined immunosuppression group; Group B, early combined immunosuppression group; PCDAI, Pediatric Crohn's Disease Activity Index; CRP, C-reactive protein; SES-CD, Simple Endoscopic Score for Crohn's Disease; MH, mucosal healing; SD, standard deviation; BMI, body mass index.

show significant differences between the two groups [Table 2]. Delta Z-scores for weight-for-age between week 54 and baseline IFX were 0.75 ± 0.89 for group A and 0.82 ± 0.64 for group B [p = 0.248]. Delta Z-scores for height-for-age between week 54 and baseline IFX were 0.10 ± 0.37 for group A and 0.11 ± 0.23 for group B [p = 0.653]. Delta Z-scores for BMI-for-age between week

Table 3. Factors associated with mucosal healing at week 54 from baseline infliximab [*n* = 76]

	Univariate analysis			Multivariate analysis with stepwise selection		
	OR	95% CI	*p*	OR	95% CI	*p*
Sex [female vs. male]	2.027	0.778–5.279	0.148			
Age	0.866	0.707–1.06	0.162			
Treatment group [group B vs. group A]	4.846	1.78–13.192	0.002	6.216	1.782–21.686	0.004
Lower GI tract involvement [L3 vs. L2]	1.415	0.243–8.245	0.67			
Upper GI tract involvement [yes vs. no]	0.266	0.1–0.711	0.008	0.245	0.07–0.858	0.028
Perianal fistula [yes vs. no]	0.381	0.147–0.9	0.048	0.278	0.083–0.929	0.038
PCDAI at baseline IFX	0.988	0.942–1.037	0.636			
ESR at baseline IFX	1.009	0.991–1.028	0.33			
CRP at baseline IFX	1.207	0.91–1.6	0.192			
Albumin at baseline IFX	0.559	0.219–1.427	0.224			
SES-CD at baseline IFX	1.071	0.977–1.175	0.142			
Deep ulceration at baseline IFX [yes vs. no]	1.267	0.499–3.216	0.619			
Clinical remission at week 14 [yes vs. no]	2.783	0.814–9.514	0.103			
Laboratory remission at week 14 [yes vs. no]	4.4	1.344–14.399	0.014			
MH at week 14 [yes vs. no]	5.702	1.957–16.614	0.001	4.183	1.245–14.06	0.02

Group A, escalated combined immunosuppression group; Group B, early combined immunosuppression group; GI, gastrointestinal; L2, colonic disease; L3, ileocolonic disease; PCDAI, Pediatric Crohn's Disease Activity Index; IFX, infliximab; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SES-CD, Simple Endoscopic Score for Crohn's Disease; MH, mucosal healing.

54 and baseline were 0.89 ± 0.88 for group A and 0.96 ± 0.74 for group B [$p = 0.381$].

3.5. Adverse events

One patient in group A, who was a primary non-responder to IFX, suffered severe anaphylaxis at his third infusion. Except for this patient, there were no other major adverse events that led to the cessation of IFX. Other adverse events that occurred during the study period were leucopoenia [$n = 10$], hair loss [$n = 6$], gastrointestinal disturbance [$n = 6$], skin rash [$N = 2$], elevation of liver enzymes [$n = 1$], and pancreatitis [$n = 1$]. No significant difference in the occurrence of adverse events was observed between the two groups [$p = 0.804$]. Dose reduction of AZA due to adverse events or elevated 6-TGN levels during the study period was observed in 16% [4/25] of patients in group A and 15% [7/48] in group B [$p = 1.000$]. One patient in group A stopped AZA and mesalazine due to pancreatitis, and one patient in group B stopped AZA due to severe leucopoenia; however, both patients were able to restart their oral medications 4 months later without any further complications. During the study period, complications of intraluminal strictures or fistulas were not observed, and none of the patients underwent surgical procedures that were related to CD.

4. Discussion

This is the first study to compare the efficacy of combined immunosuppression with IFX and AZA at both post-induction and 1 year from baseline IFX in terms of MH in children and adolescents diagnosed with moderate-to-severe luminal CD who were treated by 'escalated combined immunosuppression' versus 'early combined immunosuppression' strategies. The results of our study are novel in the fact that, although there were no statistically significant differences between the two groups regarding remission rates of all outcomes at week 14 from baseline IFX, MH rates at week 54 from baseline IFX were significantly higher in patients who were treated by an 'early combined immunosuppression' strategy initiated within 1 month from diagnosis without CS induction.

The importance of MH as a primary endpoint in evaluating treatment responses arises from the discrepancy between clinical symptoms and mucosal status in CD.[21] Likewise, in terms of remission rates between the two groups in our study, discordance was observed between clinical, laboratory, and endoscopic outcomes. The inability of clinical remission or normalization of CRP to discriminate the effect of different treatments between groups was observed in a recent post-hoc analysis of the SONIC trial.[22] In that study, statistical significance between the combination group and the IFX monotherapy group at week 26 was observed only for MH and composite outcomes that included MH, despite findings of higher remission rates for other outcomes. Thus, endoscopic evaluation in addition to the assessment of clinical symptoms and biological markers may be important for thorough detection of each patient's disease status and to determine the efficacy of treatment in real-life practice.[21,23]

In contrast to the many large-scale studies in the adult population, there are currently only a few small-scale studies that have investigated MH as an endpoint in paediatric CD patients treated with biologics.[8,9,10] According to these studies, MH rates were 23% at 2.5 months from the start of IFX treatment, and 22–42% at approximately 1 year of treatment with IFX or ADA. Compared to the results of these studies, MH rates of both groups in our study were relatively higher, showing week 14 MH rates of 32% and 51% for group A and B, respectively, and week 54 MH rates of 51% and 74% for group A and B, respectively. Disease duration was relatively longer in previous studies showing median disease durations of 12–48 months. The percentage of inflammatory behaviour was 42–75% in previous studies, while only non-penetrating, non-stricturing type [B1] was included in our study. Previous surgeries were present in 21–26% of the previous studies, while patients with previous surgeries were excluded from our study. Moreover, except for the study by Kierkus *et al.*,[7] the proportion of patients receiving combined immunosuppression was only 16–34% in the previous studies. Compared to previous studies, loss of response (LOR) in our study was also relatively lower, showing a primary non-response of 4% and secondary LOR of 9% based on dose intensification, and secondary LOR of 1% based on IFX discontinuation. According to a recent systematic review, primary non-response to anti-TNF

treatment affected 13–40% of patients, and secondary LOR to anti-TNF occurred in 23–46% of patients based on dose intensification and 5–13% of patients based on drug discontinuation rates.[24] Factors such as disease duration, characteristics, previous bowel surgery, and treatment strategies plus a possible ethnic difference in the immunogenicity to biologics may have contributed to the difference in LOR and the achievement of MH.

The underlying mechanism by which the early combined immunosuppression strategy yields superior results compared to escalated combined immunosuppression appears to be associated with the difference in disease duration between the groups. Walters *et al*.[25] have reported that early anti-TNF-α monotherapy initiated within 3 months of diagnosis was associated with significantly improved clinical outcomes, with an estimated 25% absolute improvement compared to early immunomodulator monotherapy. According to a recent post-hoc analysis of the EXTEND study, deep remission rates at 1 year were 33%, 20%, and 16% in subjects whose disease duration was ≤2 years, >2–5 years, and >5 years, respectively, among patients who had received scheduled therapy with ADA.[26] According to the aforementioned post-hoc analysis of the SONIC trial, MH rates were higher in 'early CD' patients among those under combination therapy, in which 'early CD' was defined as disease duration ≤18 months after diagnosis with no previous use of immunomodulators and biologics and no fistulas.[22] These findings suggest that the achievement of MH may be difficult in patients who are treated late in their disease course because of the progressive nature of CD leading to irreversible bowel injury, which is concordant with the concept of catching a 'therapeutic window of opportunity' in rheumatoid arthritis.[21,22,23,27] In addition, our finding that MH during combined immunosuppression is more effectively achieved by treatment with an early treatment strategy within 1 month suggests that the 'therapeutic window of opportunity in early CD' may be shorter than currently recognized, especially in paediatric CD.

Our finding that 'MH at week 14 from baseline IFX' was significantly associated with 'MH at week 54 from baseline IFX' is consistent with a Finnish study that demonstrated that 'MH at 3 months' was able to predict 'MH at 1 year' in CD patients treated by combination treatment.[28] In addition, the observation that neither SES-CD scores nor the presence of deep ulcerations on ileocolonoscopy at baseline IFX was associated with MH at both week 14 and week 54 from baseline IFX in our study implies that the mucosal response to combined immunosuppression at post-induction, rather than the severity of the mucosal status at baseline IFX, affects its status later during the treatment course. This suggests that endoscopic evaluation at post-induction with IFX could aid in predicting the disease course later during maintenance. According to a recent study, IFX trough levels at post-induction were capable of predicting sustained clinical remission without dose intensification throughout 1 year in paediatric patients with IBD.[29] Another recent study revealed that significant associations were present between serum anti-TNF agents and MH in adult patients with IBD.[30] Future studies in children with CD are required regarding the relationship between IFX trough levels and MH at both post-induction and during maintenance treatment with biologics.

Extensive pan-enteric disease and severe perianal fistulas are one of the risk factors for poor outcome and is therefore an indication for considering early treatment with IFX according to the recent consensus guideline of the European Crohn's and Colitis Organization [ECCO] and ESPGHAN on the medical management of paediatric CD.[31] Likewise, upper gastrointestinal tract involvement and the presence of perianal fistulas at diagnosis were associated with failing to achieve MH at week 54 in our study. The incidence of perianal fistulas in paediatric CD in Korea seems to be higher than that of Western countries and may be even higher in patients with moderate-to-severe luminal disease compared to mild luminal involvement.[11,32] Despite the exclusion of patients whose major problems were refractory perianal fistulas requiring IFX, there were still approximately 60% patients who presented with mild perianal fistulas and whose major problems were moderate-to-severe luminal disease. Further studies on this ethnic difference of perianal fistulas in CD are required.

Our study has some limitations. First, given that subjects and guardians chose the initial treatments, a lack of blinding and inappropriate randomization are major points that may limit the significance of the study. This is a limitation of prospective observational studies such as ours, where decisions are made when the investigators and subjects are aware of the study hypothesis. Selection bias may have also been introduced by the comparison of treatment-naïve patients versus those who had failed with conventional treatment. Thus, a possible underlying difference between the two groups regarding therapeutic response to any kind of treatment may have contributed to the difference in results, rather than the disease duration itself.

Second, some limitations are related to the period of this study, which was initiated before the publication of the recent consensus guideline of ECCO and ESPGHAN. Thus, exclusive enteral nutrition was not considered as a major therapy for induction in our study, which was initiated in 2011. Moreover, some patients in the escalated group possessing predictors of poor outcome may have required initial induction with IFX according to the ECCO/ESPGHAN guideline. Therefore, the inflammatory burden in these patients may have increased, attributing to a lesser rate of MH compared to the early treatment group. Another limitation is the small number of subjects included in our study. Although a reasonable difference in MH rate at week 14 was observed between the groups, statistical significance was not achieved. This may be due to the insufficient power originating from the small number of subjects as well as the smaller number of patients in group A. Further large-scale multicentre studies are required in the future.

In conclusion, combination therapy was more effective in terms of MH in paediatric patients with moderate-to-severe luminal CD when administered by an early combined immunosuppression strategy initiated within 1 month from diagnosis without CS induction compared to a step-up strategy initiated with CS induction. The therapeutic window of opportunity in early CD may be shorter than generally thought, especially in paediatric patients.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

BK contributed in the conception and design of the study, acquisition, analysis and interpretation of data, drafting of the initial manuscript, and critical revision for important intellectual content. SYC contributed in the acquisition, analysis and interpretation of data, and drafting of the initial manuscript. HSK contributed in statistical analysis, and drafting of the initial manuscript. KK contributed in statistical analysis, and critical revision for important intellectual content. YML contributed in the acquisition of data, and critical revision

for important intellectual content. YHC contributed in the conception and design of the study, and critical revision for important intellectual content.

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