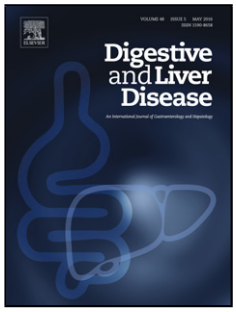

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Does Azathioprine induce endoscopic and histologic healing in Pediatric Inflammatory Bowel Disease? A prospective, observational study.

Short title: Azathioprine and mucosal healing in pediatric IBD

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Abstract

Background: The new concept of disease remission for pediatric inflammatory bowel diseases (IBD) implies the achievement of mucosal healing.

Aims: We aimed to evaluate endoscopic and histologic healing in children with Ulcerative Colitis (UC) and Crohn's disease (CD) in clinical remission after 52 weeks of Azathioprine.

Methods: From December 2012 to July 2015 we prospectively enrolled IBD children starting Azathioprine. Enrolled patients in clinical remission underwent colonoscopy after 52 weeks. Macroscopic assessment was described with Mayo score and the Simplified Endoscopic Score for UC and CD, respectively. For microscopic assessment, an average histology score was used. Data on inflammatory markers and fecal calprotectin were also collected.

Results: Fourty-seven patients were included in the analysis. Endoscopic healing was detected in 20/26 (76.9%) UC children and 10/21 (47.6%) CD patients. Median Mayo score and simplified endoscopic score were significantly decreased at week 52 ($p<0.001$; $p=0.005$). Median average histology score was not significantly different at week 52 in both diseases. Fecal calprotectin was directly correlated with simplified endoscopic score (T0: $r=0.4$, $p=0.05$; T52: $r=0.5$, $p=0.01$), but not with Mayo score. No correlation was found between endoscopic and histologic scores.

Conclusions: IBD children under azathioprine reach endoscopic healing, but not histological remission.

Key words: Children, mucosal healing, IBD, Thiopurines.

Introduction

Pediatric Inflammatory Bowel Diseases (IBD), including Crohn's disease (CD) and Ulcerative Colitis (UC), are chronic, relapsing intestinal disorders. The main goal of IBD therapeutic approach is to achieve and keep disease remission with reduction of hospitalization rates and improvement of patients' quality of life (1). The new concept of "disease remission" implies not only the resolution of symptoms, but also the achievement of mucosal healing (MH) (2). Indeed, it is well established that clinical remission does not necessarily reflect endoscopic and histologic remission (3). The ideal definition of MH is a "complete remission" better defined as an association of clinical, endoscopic and histological disease resolution together with a normalization of inflammatory markers (4). The importance of achieving MH in clinical practice is related to growing evidences that contributes to lower rates of clinical relapse, hospitalization and need for surgery (5). The real problem is the lack of a validated IBD histological activity index, which makes difficult relating IBD clinical and endoscopic scores with the histological assessment (4, 6). Therefore, to date, MH is mainly referred to the endoscopic healing (EH), while histological remission is not recommended as primary goal for therapeutic trials (5, 7). Nevertheless, it has been shown that the persistence of histological inflammation alone represents a strong predictor of clinical flares and disease complications (8, 9). Few studies demonstrated MH with the use of current medications in adult UC and CD, with limited evidences regarding biologics (10-12). Particularly, MH after azathioprine (AZA) therapy has been reported over a broad range from 16% to 70% (13). Up to now, no data have been described in pediatrics. The primary aim of our study was to evaluate the incidence of endoscopic and histologic healing in a cohort of children in clinical remission after 1 year of AZA; the secondary aim was to correlate clinical, laboratory, endoscopic and histological features.

Materials and Methods

This was a prospective, observational study, performed at the Department of Translational and Medical Science, Section of Pediatrics, University of Naples "Federico II". All children with a diagnosis of IBD needing to start AZA therapy for a relapse of disease between December 2012 and July 2015 were enrolled in the study. The diagnosis of CD and UC was confirmed by clinical, radiologic, endoscopic, and histological criteria (14). Exclusion criteria were: fistulising, perianal and symptomatic stricturing CD; other comorbidities; pregnancy; contraindications to AZA; previous treatment with thiopurines, other immunosuppressive and biologic agents. In addition, starting from 2014, after the publication ECCO guidelines on the opportunistic infections management in IBD, we started testing EBV status in all children before starting AZA, and we excluded the EBV seronegative children (15). At the enrolment, an ileo-colonoscopy was performed before starting AZA and information about demographic data, family history and IBD characteristics were recorded. For the purpose of this manuscript, disease location and phenotype were defined on the basis of Paris classification (16). Disease activity was assessed using the Pediatric Ulcerative Colitis Activity Index (PUCAI) (17) for UC and the Pediatric Crohn Disease Activity Index (PCDAI) (18) for CD. Blood samples were taken from each patient at the enrolment and at 4, 8, 12, 24 and 52 weeks after treatment's beginning to determine full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin, urea, iron, creatinine, electrolytes, pancreatic and liver function tests. Fecal calprotectin (FC) was also determined at each time point. After the ileo-colonoscopy, in line with the current guidelines exclusive enteral nutrition (EEN) based on the exclusive administration of a polymeric formula for 6-8 weeks was used as primary induction therapy in CD patients (19); in case of non compliance or non response, oral methylprednisolon (1mg/kg/day, max 40 mg/day per 4 weeks) or EEN plus steroids were used. Oral methylprednisolon was given as induction therapy for UC patients (20).

Concurrently with the induction therapies, the enrolled patients started AZA at 0.5 mg/Kg/day and weekly increased to 2-3 mg/kg/day on the basis of drug tolerance. The only concomitant maintenance treatments allowed during the study were systemic/topical aminosalicylates. Only patients in clinical remission after 52 weeks underwent a new colonoscopy and were finally included in the analysis. IBD clinical remission was defined as the maintenance of a PUCAI /PCDAI≤10 (17, 18). Clinical relapse was defined as the occurrence or worsening of symptoms, accompanied by an increase of PUCAI/PCDAI>10 points (17, 18). Primary non-response to AZA was defined when a patient, after achieving clinical remission with the induction therapy, was not able to keep PUCAI/PCDAI<10, after at least 14 weeks of optimized AZA dosage (18). Mayo endoscopic sub-score and the Simplified Endoscopic Score for CD (SES-CD) were used to assess endoscopic activity in UC and CD, respectively (21, 22). For the purposes of this manuscript the EH was defined as Mayo subscore≤1 for UC and SES-CD≤2 for CD patients. The absence of lesions at the follow-up endoscopy (Mayo and SES-CD=0) corresponded to complete endoscopic remission. An expert endoscopist (EM) performed all the procedures and prospectively assessed Mayo and SES-CD scores. During endoscopy, 4 biopsies were taken from each colonic segment and from the terminal ileum, if entered. The histologic activity was assessed by an experienced IBD pathologist (MD), who was blinded to the endoscopic features and clinical history of the patients. A scoring system, previously validated, was adopted (23, 24). The histological score combined both inflammatory changes and chronic changes involving the mucosal architecture (24). In the absence of a validated score, an average histology score (AHS) was extracted by dividing the sum of individual intestinal segmental scores by the number of intestinal segments explored, as previously reported (25). Histological healing was defined as a decrease of AHS ≥50% when compared with baseline values.

The Institutional Review Board of the University of Naples "Federico II" approved the study protocol with the registration number 239/13. Written, informed consent was obtained from parents and also from children, where appropriate.

Statistical Analysis

Variables were screened for their distribution, and appropriate parametric or non-parametric tests were adopted as necessary. For endoscopic and histologic healing, we reported the intention to treat analysis, including also the patients interrupting the study for adverse events or failing to maintain clinical remission at 52 weeks, and the per protocol analysis, only evaluating children who achieved the study completion. The Student's t-test, the ANOVA test and the Mann-Whitney test for continuous variables, the χ^2 and Fisher's exact tests for categorical variables were used, where appropriate. Multivariate conditional logistic regression analysis was used to explore the odds associated with endoscopic and histologic healing in both diseases. EH, complete endoscopic remission and histologic healing were used as dependent variables, while the effect of the baseline parameters were analyzed by a stepwise procedure. Correlations between continuous variables were evaluated through linear regression and expressed by the Spearman's correlation coefficient. Statistical significance was predetermined as $p<0.05$. Percentages were rounded to the nearest whole numbers. SPSS version 20 was used for all statistical analyses (SPSS Inc, Chicago, Illinois, USA).

Results

Sixty-one consecutive IBD children (UC: 32; CD: 29) were enrolled between December 2012 and July 2015. Fourteen (22.9%, UC/CD 6/8) patients dropped out of the study. In

details, 3 (21.4%) patients stopped AZA for severe leukopenia (<1000/mm^3), 1 (7.1%) for an acute pancreatitis and 10 (71.4%) for primary non-response. Fourty-seven patients (77%) (Median age: 12.7; M/F: 31/16; UC/CD: 26/21) reached and maintained clinical remission under AZA at the 52 weeks' follow up and were finally included in the analysis. Figure 1 shows the subjects' progression through the study. Baseline characteristics of patients completing the whole follow-up are reported in Table 1.

UC Patients

Clinical, endoscopic and laboratory characteristics of UC children at the enrolment and after 52 weeks are showed in Table 2.

Median Mayo Score at 52 weeks was significantly decreased when compared to the baseline ($p<0.001$) (Figure 2A). After 52 weeks, on the intention to treat analysis, 20 out of 32 (62.5%) children reached EH and 12 out of 32 patients (37.5%) achieved complete endoscopic remission. On the per protocol analysis, 20 out of 26 (76.9%) patients reached EH defined as a Mayo score≤1, while complete endoscopic remission was achieved in 12 out of 26 (46.1%) patients. Median AZA dosage was not different between patients reaching and not reaching EH [2.2 mg/Kg/day (range 2-3) vs 2.1 (range 2-3); p=1)]. EH was not significantly different when comparing UC children treated with AZA alone [9/12 (75%)] versus patients with AZA and 5-ASA [11/14 (78.6%); p=1]. EH was not associated with median disease duration [13 months (mths) (3-66) vs 10.5 mths (6-39); p=0.6)].

Median AHS was not significantly different at weeks 52 when compared with baseline (p=0.8) (Figure 2B). Histological healing defined as an AHS reduction ≥ 50% was reached in 6/32 (18,7%) on the intention to treat analysis and in 6 out of 26 (23.1%) on a per protocol basis. In the remaining patients, a mild improvement of AHS was observed in 6/26 (23.1%), 6 (23.1%) children did not show any change, and worsening was observed

in 8 cases (30.8%). None of the patients achieved a complete histological remission. When considering independently each histologic variable, the median score of mucosal architecture in ascending colon, transverse colon and rectum was the only parameter significantly improved when compared to the baseline [1.5 (range 1-3) vs 1 (range 0-3), p=0.002; 2(0-3) vs 1 (1-3), p=0.003; 2(0-3) vs 1.5 (0-3); p=0.005, respectively]. No differences were detected regarding lamina propria cellular infiltration.

Median PUCAI score at 52 weeks was significantly decreased respect to the baseline (p<0.001). Disease extension, based on Paris classification, was significantly improved at week 52, with a decrease of pancolitis frequency (p<0.001). At 52 weeks, a significant improvement of serological and inflammatory parameters was observed when compared to the baseline, including hemoglobin (p=0.001), albumin (p=0.004), platelets (p=0.001), CRP (p=0.06), ESR (p=0.006) and FC (p=0.03).

CD Patients

Clinical, endoscopic and laboratory characteristics of CD children at the enrolment and after 52 weeks are showed in Table 3.

Median SES-CD at 52 weeks was significantly decreased when compared to the baseline (p=0.005) (Figure 2C). At week 52, on the intention to treat analysis, 10 children out of 29 (34.4%) reached EH, while only 2 out of 29 (6.8%) showed a complete endoscopic remission. On the per protocol analysis, 10 patients out of 21 CD (47.6%) reached EH measured through SES-CD≤2 and complete endoscopic remission was achieved in 2 out of 21 (9.5%) patients. EH did not result to be significantly associated with different induction therapies when comparing children with a SES-CD≤2 (Steroids: 8; EEN: 2; EEN+Steroids: 1) vs remaining patients [(Steroids: 8; EEN: 1; EEN+steroids: 1) ,

p=0.8]. EH was not significantly different when comparing children treated with AZA alone [4/10 (40%)] versus children with AZA and 5-ASA [6/11 (54.5%); p=0.6]. Median AZA dosage was not different between patients reaching and not reaching MH [2.3 mg/Kg/day (range 2-3) vs 2.3 (range 2-3); p=1)]. EH was not associated with median disease duration [11.5 mths (2-96) vs 16 mths (4-92); p=0.2)].

Median AHS was not significantly different at weeks 52 when compared with baseline (p=0.7) (Figure 2D). Histological healing was reached in 2/29 (6.8%) children on the intention to treat analysis and in 2 out of 21 (9.5%) on a per-protocol basis. In the remaining children, a mild improvement of AHS was observed in 6 out of 21 patients (28.6%), 6 (28.6%) subjects did not show any change and worsening was observed in 7 cases (33.3%). None of patients achieved a complete histological remission. When considering independently each histologic variable, no improvement was detected after AZA therapy compared to baseline.

Median PCDAI score at 52 weeks was significantly decreased respect to the baseline (p<0.001). At week 52 a significant improvement of serological and inflammatory parameters was observed when compared to the baseline, including hemoglobin (p=0.01), albumin (p<0.001) and FC (p=0.001).

Multivariate analysis

Ulcerative Colitis

In UC children, a baseline fecal calprotectin value >300 ug/g was the only independent factor inversely associated with the reach of complete endoscopic remission (Mayo subscore=0) (Adjusted OR=0.72; 95% Confidence Intervals: 0.12-0.94; p=0.03). None of

the analyzed variables resulted to be significantly associated with EH (Mayo subscore<1) and histologic healing.

Crohn's disease

In CD patients, the only significant factor negatively associated with the reach of EH (SES-CD<2) was the presence of ileo-colonic disease (L3) at the enrollment (Adjusted OR: 0.9; 95% Confidence Intervals: 0.4-1; p=0.01). None of the analyzed variables resulted to be significant associated with complete endoscopic remission (SES-CD=0) and histologic healing.

Spearman correlations

Fecal Calprotectin and serological variables

FC was directly correlated with SES-CD at week 0 and week 52 (T0: r=0.4, p=0.05; T52: r=0.5, p=0.01). No correlation was found between FC and Mayo score at T0 and at T52 (T0, r=-0.02, p=0.9; T52, r=0.1, p=0.3). We didn't find any correlation between FC and the AHS scores at week 0 and week 52 (FC and UC AHS: T0, r=0.1, p=0.6; T52, r=-0.1, p=0.4; FC and CD AHS: T0, r=0.09, p=0.6; T52, r=0.4, p=0.08). None of the other serological variables correlated with endoscopic and histologic scores.

Disease Activity Indexes

PCDAI did not correlate with SES-CD at week 0 and 52 (r=-0.1, p=0.6; r=0.2, p= 0.3, respectively). No correlation was found between PUCAI and Mayo score at week 0 and at week 52 (r=-0.1, p=0.4; r=0.3, p=0.7).

MH and Histology

SES-CD and AHS were not correlated both at week 0 and week 52 (r=0.1, p=0.4; r=0.2, p=0.3, respectively). No significant correlation was found between Mayo endoscopic score and AHS at week 0 and week 52 (r=-0.04, p=0.8; r=0.3, p=0.09, respectively).

Discussion

To the best of our knowledge this is the first pediatric study investigating the association between clinical remission and both endoscopic and histological healing in IBD children under AZA. Although clinical remission after 1 year was associated with the achievement of EH in 76.9% UC children and in 46.9% CD patients, none of our children showed histological healing.

To date the efficacy of AZA for the induction of EH has only been evaluated in few adult studies. D'Haens and colleagues published 2 different studies. In the first paper, 15 CD patients with post-operative recurrence of ileitis, treated with AZA, reached EH in approximately 40% of cases, after a median time of 18 months (26). In the 2nd study, 54% of CD patients showed healing of the mucosal lesions located in the ileum and 70% healing of the colonic lesions, after a median time of treatment of 2 years with AZA (27). More recently, Mantzaris et al., randomized 77 patients with steroid-dependent CD to receive either budesonide or AZA as maintenance treatment for 1 year. On the intention-to-treat analysis and considering complete EH, the percentage of patients with was 58% (25). Differently from the previous studies, the SONIC, which compared AZA, infliximab and the combination therapy in moderate to severe CD, demonstrated that only 16% of patients receiving AZA achieved complete EH (28). In our study population, on the per-protocol analysis, 46.8% of CD children reached EH defined as a SES-CD≤2 and only 9.5% achieved a complete endoscopic remission. These findings distinctly underscore

that AZA is able to improve SES-CD in a small percentage of children and rarely a complete endoscopic remission is reached. Not surprisingly, the presence of a major extent of disease, such as ileo-colonic location, resulted the only independent factor negatively associated with EH in CD. Differently from CD, data on UC are more encouraging: 76.9% of UC patients achieved MH defined as Mayo score ≤1 and 46.2% obtained a complete endoscopic remission. To our knowledge, only 1 RCT by Ardizzone et al. evaluated the efficacy of AZA on UC endoscopic healing. AZA induced clinical and endoscopic remission in 55% of patients (29).

Recently, an international committee defined the STRIDE (*Selecting Therapeutic Targets in IBD*) program with the purpose to achieve a consensus on evidence-based treatment goals for IBD (5). The panel stated that EH has to be considered the primary target for both UC and CD. Due to the poor evidences, histology was not included within the main therapeutic goals (5). Nevertheless, some recent papers stressed the importance of persistent histological inflammation, which may be a better predictor of future clinical relapse than macroscopic appearance alone (30-31). Particularly, Zenlea et al. described a prospective cohort of 179 UC patients in clinical remission for 12 months and demonstrated that histology grade was the only independent factor associated with clinical relapse (9). Furthermore, several studies in adults have reported that microscopic inflammation persists in 16-100% of endoscopically quiescent UC (32) and also occurs in 25-37% of CD patients with clinical and endoscopic remission (33). In agreement with this literature, we demonstrated that EH is not necessarily reflective of histological healing. Indeed, we found significant lower percentages of histologic healing and no correlation between endoscopic and histologic scores. The correlation between endoscopy and histology has been poorly addressed in pediatrics, mainly due to the lack of a validated histologic score. Recently, Santha et al. retrospectively investigated endoscopic and histological healing among a cohort of IBD children (34). The authors included in the

analysis 104 patients diagnosed from 2010 to 2016 and concluded that the rate of histological healing was lower than endoscopic remission (34). A systematic review published in 2013, including adult and pediatric studies, stated that only about 30% of patients is able to reach a true histological remission (35). The most disappointing finding in our population under AZA is that none of the children reached a complete histologic healing. We only found a moderate improvement of mucosal architecture in UC patients, while the histological alterations of CD remained definitely unchanged. Whether this may be due to the strict criteria of our pathologist or to the lack of a validated score need to be further addressed. However, we hypothesize that AZA may only give a superficial healing and that it could not be sufficient to keep a long-term remission in IBD children.

The importance of MH and the consequent need for more endoscopic evaluations have led to new practical and ethical issues. When should we look for MH? How many endoscopies are we allowed to perform in children? Repeating endoscopic procedures in children is unpleasant and possibly painful, besides being time-consuming and expensive. In addition, as further confirmed by our results, clinical activity indexes, are not perfectly correlated with MH. For these reasons, there is a growing interest in researching new possible surrogate markers that may reflect the severity of mucosal inflammation. FC seems to be the most reliable candidate. As a matter of fact, it has been reported a moderate to good correlation between FC and endoscopic scores in adult studies (36-37). Two different pediatric studies evaluated FC as a proxy of mucosal status (31, 38). In line with this literature, in our study population we found a direct correlation between SES-CD and FC both at the enrolment and at week 52. This result once more underscores the potential role of FC monitoring in quiescent CD. Furthermore, a higher value of FC (>300) at enrollment resulted to be negatively associated with the reach of complete endoscopic remission at 52 weeks in UC children.

With regards to the clinical activity indexes, we confirmed that PCDAI is far to be correlated with MH (31). Surprisingly, we did not find any correlation between PUCAI and the Mayo sub-score. Adult's paper described a good correlation between UC activity indexes and endoscopic scores (21, 39). In children, Turner et al. firstly reported the correlation of PUCAI with the Full Mayo Score (17). To our knowledge, only recently Kerur B et al retrospectively demonstrated a direct correlation between PUCAI and the sole Mayo rectal endoscopic sub-score (40). Our results underline the need for specific pediatric score, rather than a lack of correlation between PUCAI and macroscopic activity. Indeed, Mayo endoscopic score has some well-known limitations, including the lack of distinction between deep and superficial ulcerations (21). Furthermore, differently from SES-CD, Mayo sub-score does not allow a quantitative evaluation of each different colonic segment. This last characteristic may have affected the correlation with the PUCAI, particularly in a rather small population, such as ours.

It is acknowledged that our study is not without limitations. The major limitation is the lack of standardized pediatric endoscopic and histological scores. Secondly, we did not measure AZA metabolites, including 6-thioguanine or 6-methilmercaptopurine. Nevertheless, we used the currently recommended AZA dosages with a gradual increase and a strict monitoring of drug tolerance; this should have adequately restricted the possibility of a non-optimized therapy. Finally, the endoscopic evaluations were not performed in blind and we cannot completely exclude an overestimation of the mucosal improvement.

In conclusion, although AZA is able to induce clinical remission and endoscopic healing in a variable percentage of patients, a complete endoscopic remission is rarely obtained. As a matter of fact, AZA is not able to induce histologic remission. Taking into account the negative predictive data of persistent histological inflammation, we

hypothesize that AZA may not be sufficient to keep a long-term remission in IBD children, particularly in CD. Larger studies are mandatory to assess the impact of endoscopic and histological healing on disease course.

Conflict of Interest Statement: Annamaria Staiano served as investigator and member of advisory board for the following companies: D.M.G, Valeas, Angelini, Miltè, Danone, Nestlé, Sucampo, Menarini. Erasmo Miele served as speaker, as investigator and member of advisory board for the following companies: Abbvie, Angelini, Bioprojet, Ferring, Menarini, Milte, Valeas. The remaining authors have no conflict of interest to declare.

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Figures Legend.

Figure 1: Flow diagram of the subjects' progression through the study.

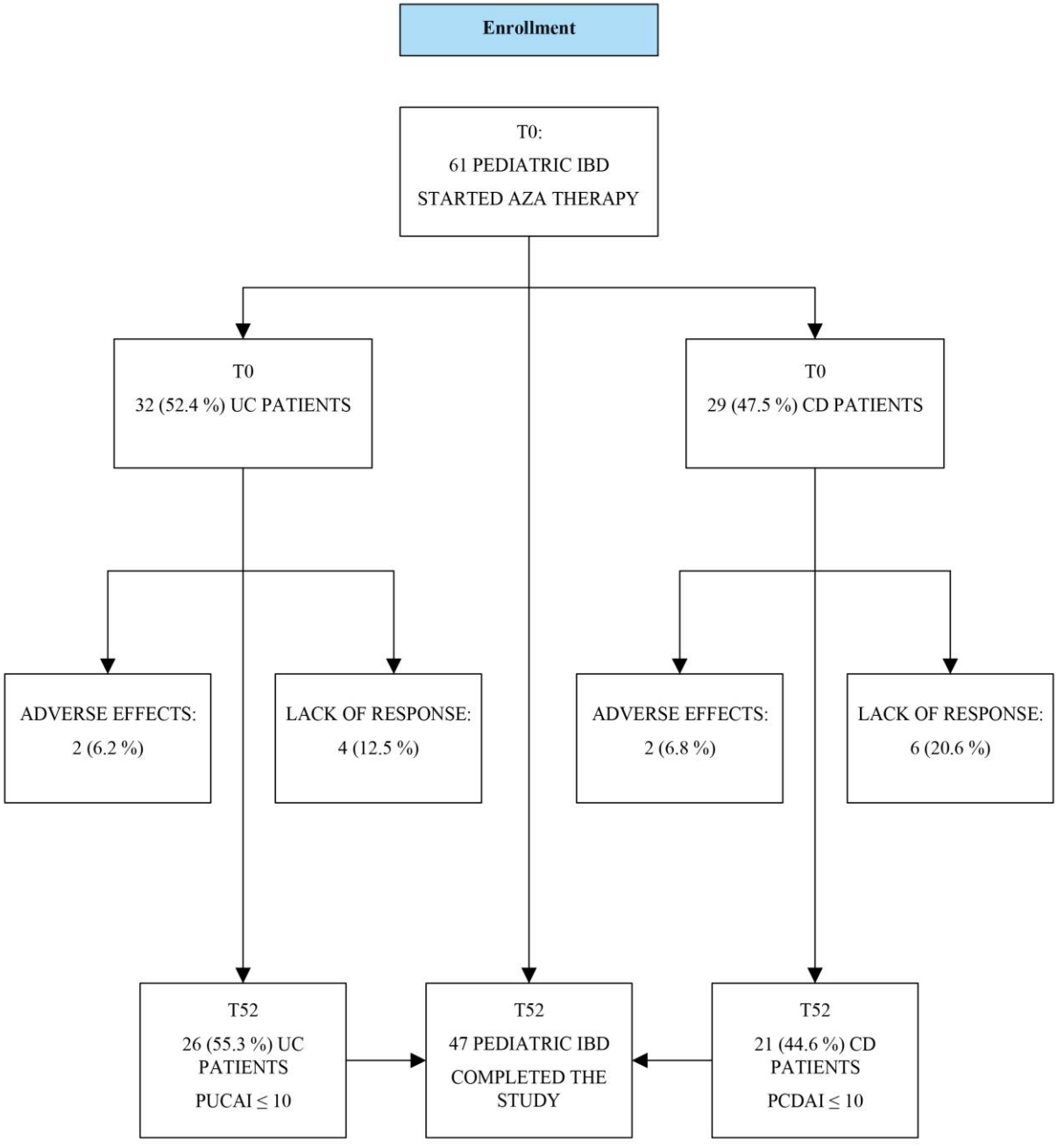

Figure 2: Mayo Endoscopic Score (A) and Average Histology Score (B) at enrolment and after 52 weeks of Azathioprine Therapy in 27 children affected by Ulcerative Colitis (p=<0.001; p=0.8, respectively); Severity Endoscopy Score (C) and Average Histology

Score (D) at enrolment and after 52 weeks of Azathioprine Therapy in 23 children affected by Crohn's disease (p=0.005; p=0.7, respectively).

CD: Crohn's disease; UC: Ulcerative Colitis.

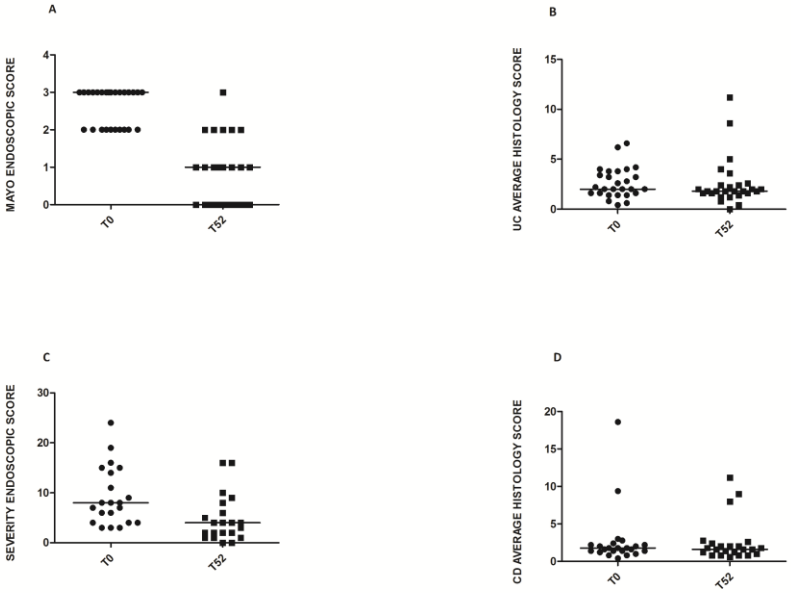

Table 1. Baseline characteristics of 47 consecutive IBD pediatric patients starting Azathioprine therapy.

Characteristics	**UC (n=26)**	**CD (n=21)**	**p**
Median age (years, range)	12.7 (4-15.9)	13.5 (3-16.7)	0.9
Male gender (n, %)	18 (69.2)	13 (61.9)	0.7
Median age at diagnosis (years, range)	12 (2-14.5)	11 (2-16.4)	0.4
Median disease duration (months, range)	12.5 (3-66)	13 (2-96)	0.5
Median PUCAI/PCDAI at diagnosis	45 (15-85)	35 (10-60)	NA
Median Azathioprine dosage	2.2 (2-3)	2.3 (2-3)	0.2
Paris classification at diagnosis (n, %)			
UC			
Proctosigmoiditis (E1)	4 (15.5)	-	NA
Left-sided colitis (E2)	3 (11.5)	-	
Extensive colitis (E3)	3 (11.5)	-	
Pancolitis (E4)	16 (61.5)	-	
CD			
Ileum only (L1)	-	2 (9.5)	NA
Colon only (L2)	-	6 (28.5)	
Ileum and colon (L3)	-	13 (61.9)	
Upper gastrointestinal tract (L4a)	-	4 (19)	
CD Behaviour at diagnosis (n, %)			
B1	-	21 (100)	NA
B2	-	0	
B3	-	0	
Induction therapy at diagnosis (n, %)			
Exclusive Enteral Nutrition	-	16 (76.1)	NA
Steroids	18 (70.3)	4 (19)	

5-ASA	20 (76.9)	3 (14.2)	

5-ASA: Aminosalicylates
IBD: Inflammatory Bowel Disease
CD: Crohn's disease
UC: Ulcerative Colitis
NA: not applicable
PCDAI: Pediatric Crohn's Disease Activity Index
PUCAI: Pediatric Ulcerative Colitis Disease Activity Index

Table 2. Clinical, Endoscopic and Laboratory Characteristics Before and After 1 Year of Azathioprine in 26 Consecutive Children Affected by UC.

Characteristics	**Week 0**	**Week 52**	**p**
Clinical Parameters			
Median PUCAI *(range)*	37.5 (10-80)	0 (0-5)	<0.001
Disease activity based on PUCAI *(n, %)*			
Severe (≥65)	4 (15.3)	0	0.1
Moderate (35-64)	20 (76.9)	0	<0.001
Mild (10-34)	2 (7.6)	0	0.2
Remission (<10)	0	26 (100)	<0.001
Endoscopic parameters			
Median Mayo Endoscopic Score *(range)*	3 (2-3)	1 (0-3)	<0.001
Children with Mayo Score=3 *(n, %)*	16 (61.5)	1 (0.4)	<0.001
Median AHS *(range)*	2.1 (0.4-6.6)	1.8 (0-11.2)	0.8
Paris Classification *(n, %)*			
Proctosigmoiditis (E1)	2 (7.7)	4 (15.3)	0.6
Left-sided colitis (E2)	1 (3.8)	2 (7.6)	1
Extensive colitis (E3)	4 (15.4)	8 (19.2)	1
Pancolitis (E4)	19 (73.1)	0 (3.8)	<0.001
Complete endoscopic remission	0	12 (46.2)	<0.001
Laboratory parameters			
Median Hemoglobin (g/dl) *(range)*	12.1 (6.6-15.5)	12.9 (11-15.6)	0.001
Median Albumin (g/dl) *(range)*	4.0 (2.6-4.7)	4.4 (3.5-5.2)	0.004
Median Platelet count (10^5/mcl) *(range)*	3.6 (2-6)	2.9 (1.4-4.5)	0.001
Median CRP (mg/dl) *(range)*	0.98 (0.2-49.6)	0.4 (0.2-7.8)	0.06
Median ESR (mm/h) *(range)*	10 (2.6-24)	3 (1-16)	0.006
Median Fecal calprotectin (ug/g) *(range)*	500 (50-3000)	146.5 (15-500)	0.03
Re-induction therapy (n, %)			
Steroids	26 (100)	-	NA

Concomitant Maintenance therapy (n, %)			
5-ASA Therapy	26 (100)	15 (57.7)	0.001
Systemic	26 (100)	9 (34.6)	0.001
Topical	0	6 (23)	0.02

5-ASA: Aminosalicylates
AHS: Average Histology Score
CRP: C-reactive protein
ESR: erythrocyte sedimentation rate
NS: Not significant
PUCAI: Pediatric Ulcerative Colitis Disease Activity Index

Table 3. Clinical, endoscopic and laboratory characteristics before and after 1 year of Azathioprine in 21 consecutive children affected by CD.

Characteristics	**Week 0**	**Week 52**	**p**
Clinical Parameters			
Median PCDAI *(range)*	25 (10-50)	0 (0-7.5)	<0.001
Disease activity based on PCDAI *(n, %)*			
Moderate-severe (>30)	18 (85.7)	0	<0.001
Mild (10-30)	3 (14.3)	0	0.2
Remission (<10)	0	21	<0.001
CD Behaviour *(n, %)*			1
B1	20 (87)	20 (87)	
B2	3 (13)	3 (13)	
B3	0	0	
Endoscopic Parameters			
Median SES-CD *(range)*	8 (3-24)	4 (0-16)	0.005
Median AHS *(range)*	1.8 (0.4-18.6)	1.8 (0-11.2)	0.7
Paris classification *(n, %)*			
Ileum only (L1)	5 (23.8)	6 (28.6)	1
Colon only (L2)	5 (23.8)	5 (23.8)	1
Ileum and colon (L3)	11 (52.4)	8 (38.1)	0.1
Upper gastrointestinal tract (L4a)	2 (9.5)	0	0.4
Complete endoscopic remission	0	2 (9.5)	0.4
Laboratory Parameters			
Median Hemoglobin (g/dl) *(range)*	11.2 (8.6-14.6)	12.7 (9.5-17.7)	0.01
Median Albumin (g/dl) *(range)*	3.8 (2.5-4.7)	4.5 (2.7-5.3)	<0.001
Median Platelet count (x10^5/mcl) *(range)*	3.5 (0.4-7.6)	3 (1.4-4.5)	0.2
Median CRP (mg/dl) *(range)*	3.59 (0.2-64.1)	0.6 (0.2-34.4)	0.1
Median ESR (mm/h) *(range)*	11.5 (2-60)	6 (1-27)	0.09
Median Fecal calprotectin (ug/g) *(range)*	483 (200-1470)	139 (15-500)	0.001
Re-induction therapy (n, %)			

Steroids	16 (76.2)	-	NA
EEN	3 (14.3)	-	NA
Steroids+EEN	2 (9.5)	-	NA
Concomitant maintenance therapy (n, %)			
5-ASA therapy	17 (80.9)	11 (52.3)	0.1

5-ASA: Aminosalicylates; AHS: Average Histology Score; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NS: Not significant; PCDAI: Pediatric Crohn's Disease Activity Index; SES-CD: Simplified Endoscopic Score-Crohn's Disease.