Digestive and Liver Disease xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

Does Azathioprine induce endoscopic and histologic healing in pediatric inflammatory bowel disease? A prospective, observational study

Francesca Paola Giugliano[a,1], Caterina Strisciuglio[a,b,1], Massimo Martinelli[a,1], Marialuisa Andreozzi[a], Sabrina Cenni[a], Severo Campione[c], Maria D'Armiento[c], Annamaria Staiano[a], Erasmo Miele[a,*]

[a] Department of Translational and Medical Science, Section of Pediatrics, University of Naples "Federico II", Naples, Italy
[b] Department of Woman, Child and General Specialized Surgery, Second University of Naples, Naples, Italy
[c] Department of Biomorphological and Functional Sciences, Section of Pathology, University of Naples "Federico II", Naples, Italy

ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form 16 October 2017
Accepted 20 October 2017
Available online xxx

Keywords:
Children
IBD
Mucosal healing
Thiopurines

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.

1. 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

* Corresponding author at: Department of Translational Medical Science, Section of Pediatrics, University of Naples "Federico II", Via S. Pansini, 5, 80131 Naples, Italy.
E-mail address: erasmo.miele@unina.it (E. Miele).
[1] Drs Giugliano, Strisciuglio and Martinelli participated equally in this study and therefore should be considered all three as first authors.

https://doi.org/10.1016/j.dld.2017.10.017
1590-8658/

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.

2. 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 of 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 (1 mg/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 $\geq 50\%$ 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.

2.1. 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).

3. 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. Fig. 1 shows the subjects' progression through the study. Baseline characteristics of patients completing the whole follow-up are reported in Table 1.

3.1. 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 ($p < 0.001$) (Fig. 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

Please cite this article in press as: Giugliano FP, et al. Does Azathioprine induce endoscopic and histologic healing in pediatric inflammatory bowel disease? A prospective, observational study. Dig Liver Dis (2017), https://doi.org/10.1016/j.dld.2017.10.017