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"THE CONTRIBUTION OF PATHOLOGICAL EVALUATION OF MUCOSAL HEALING IN THE MANAGEMENT OF PATIENTS AFFECTED BY INFLAMMATORY BOWEL DISEASES"

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ABSTRACT

Introduction: Mucosal Healing (MH) is becoming an increasing tool in the management of patients affected by inflammatory bowel diseases and is set as the endpoint of a therapy in clinical trials, especially for the recently introduced biological agents.

MH is evaluated by direct investigation instruments and in most clinical trials these are represented by endoscopy.

There is no definition of MH in pathology and there are no established evaluation criteria of MH among pathologists.

Aim of this study is to find a pathological definition of MH and a pathological score as useful as endoscopy scores to the clinical management.

Methods: a population of 51 patients in pediatric age is selected, affected by both Crohn's disease and ulcerative colitis, and is treated for 1 year with Azathioprine. All patients undergo colonoscopy with biopsies at the beginning and at the end of the therapy. Patients are followed for 2 years after the end of the trial and some of them have biopsy follow up. Biopsies are evaluated with two different scores, the Combined Architectural and Inflammatory Score and the Activity Score in order to evaluate MH. An immunohistochemical analysis is also performed. Results are correlated with Endoscopy and follow up.

Conclusions: Among the two scores, the Activity Score exhibited higher correlation with endoscopy, demonstrating the usefulness of pathological MH. Moreover, both scores produced a significant prediction of release during follow up.

1. INTRODUCTION

1.1 GENERAL UPDATES ON INFLAMMATORY BOWEL DISEASES

Inflammatory bowel diseases (IBD) are gastro-intestinal chronic diseases characterized by chronic inflammation and variable clinical courses, consisting of releases and relapses. IBD group two distinct entities, Ulcerative colitis (UC) and Crohn's disease (CD), differing in several pathological features. Firstly, UC involves the colon starting from the rectum and continuously variable portions of the colon, while CD can affect the whole digestive tube and typically goes discontinuously, with ileum and colonic disease being the most frequent. Secondly, UC is a disease of the intestinal mucosa and submucosa, while CD is a transparietal disease. These differences imply a divergent biopsy approach and differences in pathological evaluation.

IBD in industrial world account for about 6 cases incidence every 100.000 individuals, with a peak in young adults between 15 and 30 year old. Middle and north Europe, USA, Canada, Australia, New Zealand are high incidence areas, while Mediterranean sea and middle east are low incidence areas. Ashkenazi Jews count the highest incidence in the world.

Causes and pathogenesis of IBD involve the interaction between environment, probably also microorganisms, and genetic factors that elicit an immune-mediated chronic inflammatory response primarily located in the intestinal mucosa.

Interestingly, cigarettes smoking is protective against UC (1).

Both UC and CD are treated with anti-inflammatory drugs and, when necessary, with surgery. Inadequacy of medical therapy, obstruction and other complications are major indications for surgery. Drugs approach is based on a growing scale of dose and medication, among 5-ASA, steroids, immune-modulators and biological agents (2).

These drugs work primarily as immunosuppressant. Biological agents work as monoclonal antibodies against the cytokine TNFα that is involved in the maintenance of chronic inflammation.

Diagnosis is usually based on clinical symptoms and signs, laboratory data, endoscopy with biopsies. Patients are then treated and followed mainly on the base of clinical indices and laboratory data, eventually with endoscopy (3).

In this setting, the most important decisions are on the therapy, looking for the longest release from the disease and the less inconveniences for the patient (4).

1.2 PATHOLOGY OF INFLAMMATORY BOWEL DISEASE

Pathologists are contributive in management of IBD patients in establishing the diagnosis, often reached after a long and complex clinical history, by evaluation of intestinal biopsies obtained during colonoscopy. During relapses patients undergo colonoscopy and biopsies are evaluated, usually to exclude superinfections and to confirm active inflammatory disease. In long time conditions, patients are carefully controlled for the development of intestinal dysplasia and eventually carcinoma. When patients undergo surgery, surgical specimens are also evaluated for the same indications.

Pathological features of IBD in intestinal biopsies consist of architectural modifications and inflammation of the mucosa. Architectural modifications include glandular atrophy, branching and distortion. Inflammation consist of mononuclear cells increase in the lamina propria, especially lymphocytes and plasma cells; typically, they locate also at the base of the crypts, differing from other inflammatory conditions. Eosinophils are also part of the inflammatory infiltrate. Aggression of the epithelium by neutrophil granulocytes is defined as activity. Epithelioid granulomas are typical of CD.

1.2.1 PATHOLOGY OF INFLAMMATORY BOWEL DISEASE IN CHILDREN

Pediatric patients, ranging from 0 to 18 years old, can be equally affected by IBD as adults.

There are differences in pathology between adults and children: generally, all features of chronicity are less apparent. A second important feature is that UC can manifest with rectal sparing and some degree of patchiness. Finally, granulomas in CD are more evident in children then adults (5-6).

These variations between adults and children patients can make it harder the distinction separating IBD and other inflammatory conditions, or separating CD and UC.

1.3 MUCOSAL HEALING

Mucosal Healing (MH) can be merely defined as the disappearance of intestinal lesions after the therapy.

Management of patients affected by IBD was based primarily on getting clinical remission as a goal of the therapy, before endoscopy was largely and easily available. The use of endoscopy made it possible the evaluation of MH at the end of the therapy and herewith the effectiveness of the therapy.

The introduction of MH has changed clinical management of patients having with it a more reliable indicator of disease release in comparison with clinical signs and symptoms and laboratory data (7-8).

The above definition of MH and its use in clinical practice has been firstly developed for endoscopy (9).

However, a universally shared definition of MH among endoscopists is still difficultly reached, ranging it from the absence of signs of activity to the disappearance of intestinal lesions. Moreover, MH should be evaluated in the setting of a specific therapy, due to possible changes in its definition between drugs and also the timing of endoscopy. Finally, so used, MH is not graded: either MH is reached or it is not (10).

The validation of MH has been differently considered for UC and CD, with more impressive results for UC (11).

MH has been used in different studies and it has been widened as a concept to clinical and laboratory indices, as indirect revelator. Pediatric ulcerative colitis activity index (PUCAI) and pediatric Crohn disease activity index (PCDAI) are current used for clinical indices, while the dosage of Fecal Calprotectin, among others, is employed between laboratory data (12-14).

However, as a manifestation of the mucosal state, it is best used by direct methods of revelation, therefore endoscopy and pathology.

MH has been presented as a possible goal of a treatment, proposing it as the endpoint of a therapy (10). In this setting, the contribution of MH to clinical management of patients affected by IBD can be particularly promising.

The recent introduction of new drugs, particularly biological agents, has promoted the search for the MH to judge the efficacy of the therapy, making it the goal of the treatment (15-17).

Up to date, MH is endorsed in endoscopy as the goal of the therapy during clinical trials (18).

1.3.1 MUCOSAL HEALING IN PATHOLOGY

MH as a direct measure of intestinal mucosa can be evaluated by pathology.

When endoscopy is performed, it is easily combined with mucosal biopsies.

Colonic biopsies together with endoscopy are direct evaluations of the disease. Endoscopy and pathology are complementary; moreover, as stated more then 50 years ago, endoscopic healing may hide histological active disease (19).

While laboratory data and endoscopy scores have easily been adapted for a definition of MH in clinical trials, particularly in the setting of UC, pathological definition of MH is going to be reached only after diverse complications (18): disease and activity scores are easier made for UC then CD, given the localization of the disease and the intestinal discontinuity and parietal localization of CD. Scores present in the literature have been often abandoned due to the hard reproducibility and the scarce contribution to clinical management (20-23). Reproducibility is also scarce among pathologist in describing and measuring elementary lesions.

Endoscopy compare whole colonoscopic images while pathologists work on multiple biopsies from different colonic sites. They should assess a MH for each biopsy examined, compared with the preceding biopsy, and then comparing the whole result for all biopsy sites submitted.

All these factors made it difficult a definition of MH in pathology, a comparison of it to endoscopic MH and its use in clinical trials.

So, non consensus and definition of MH in pathology existed at the beginning of our investigation (18).

Finally, MH should be evaluated in the setting of a specific therapy: different drugs could reach different MH and could imply different timing of the evaluation (18).

2. AIMS OF THE RESEARCH

The investigation had the purpose to explore the role of pathological MH in a pediatric population of patients affected by IBD, both UC and MC, followed during 1 year of therapy with Azathioprine, an immune-modulator drug.

Intention of the study was to find a definition of MH in pathology.

Firstly, we have investigated different definitions and grading scores of MH in pathology, thereafter we have matched these pathological scores with endoscopy.

Secondly, the children were monitored for 2 additional years in order to compare MH with clinical courses.

3. MATERIALS AND METHODS

3.1 PATIENTS ENROLLMENT

Fifty-one patients in pediatric age affected by IBD were selected by pediatric colleagues of the University Hospital Federico II of Naples.

Twenty-four patients were affected by CD; their age at the first colonoscopy was between 3 and 16 year old (mean age 11.7 median age 11 mode 13). Four children were younger then 10.

Twenty-seven patients were affected by UC; their age at the first colonoscopy was between 3 and 15 year old (mean age 11.5 median age 12 mode 14). Five children were younger then 10.

All patients had established diagnosis of IBD, either CD or UC, with colonic involvement, and all patients were eligible for starting a therapy with a immune-modulator drug.

The time of the clinical study ranged from 2012 to 2015.

All patients recruited underwent colonoscopy with mucosal biopsies from every colonic segment, including terminal ileum, before starting Azathioprine therapy for 1 year. Thereafter, a new colonoscopy was performed and biopsies were taken in the same places of the first examination.

Written informed consent was received from all parents of pediatric patients and the Institutional Review Board of the University of Naples Federico II authorized the clinical study.

3.2 BIOPSIES EVALUATION

Each patient had biopsies from at least 5 different intestinal areas (terminal ileum, colon ascending, colon transverse, colon descending, rectum).

All biopsies were submitted to Pathology Section and processed according to diagnostic purposes.

Tissue was formalin fixed, dehydrated, paraffin embedded and 4 micron cut and mounted on glass slides.

Slides were stained with haematoxylin-eosin and were studied on light microscopy (LEICA DM1000) by two pathologists.

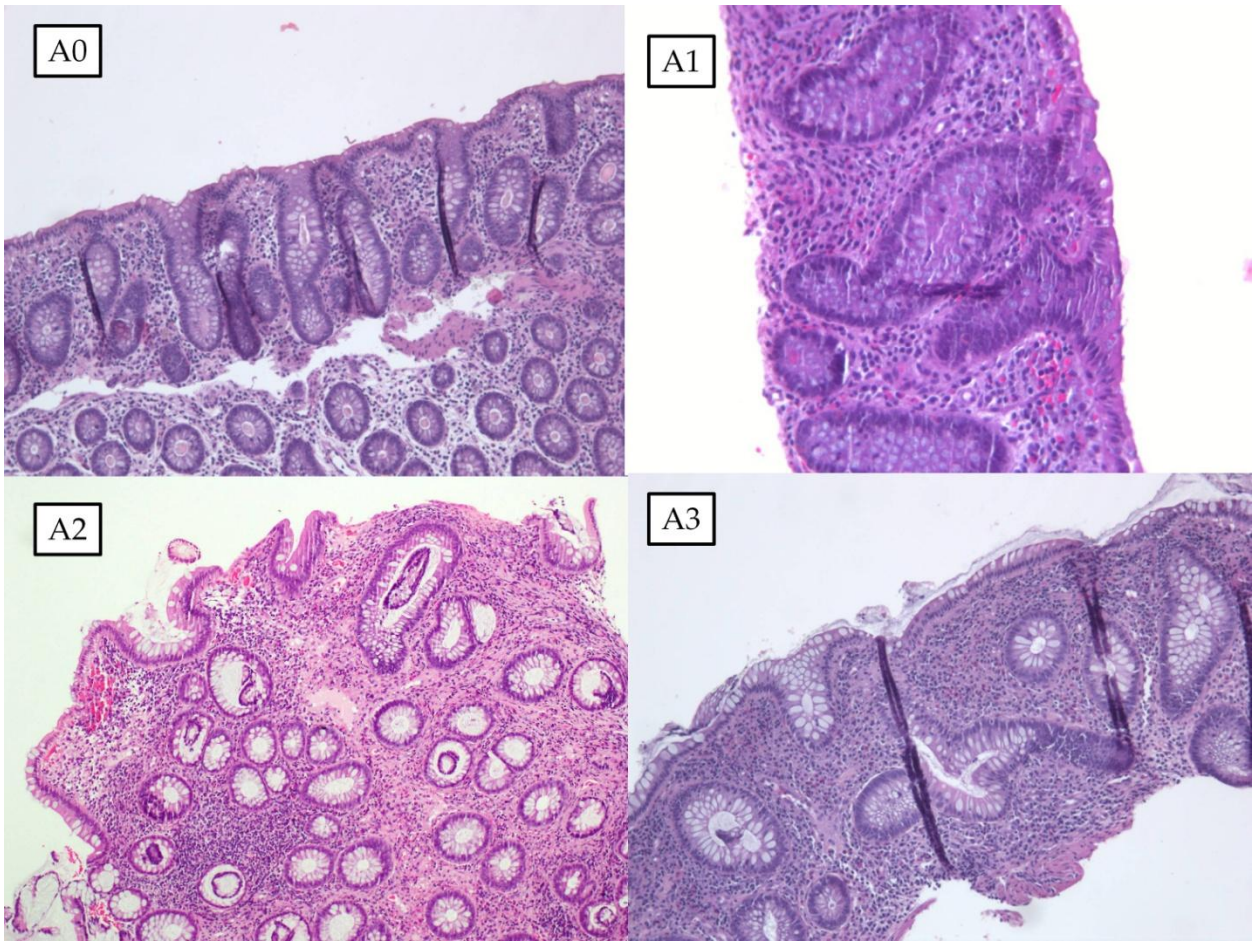
MH has been evaluated producing two independent scores and an immunohistochemistry analysis.

3.2.1 FIRST MH SCORE

For the first score, the combined architectural and inflammatory score (FIRST SCORE - CAIS), we proceeded in this way: a semi-quantitative twofold score was designed, separating architecture modifications and inflammation severity. Both scores ranged from 0 to 3, where 0 stated no alterations, 1 mild, 2 moderate and 3 severe alterations. Architecture score was given combining crypt branching, distortion, atrophy and mucin depletion. Inflammatory score was given combining the intensity of lymphoid infiltrate in the lamina propria with the presence of additional findings, such as basal plasmacytosis, increase of eosinophils in the lamina propria, presence of granulomas (CD) and activity.

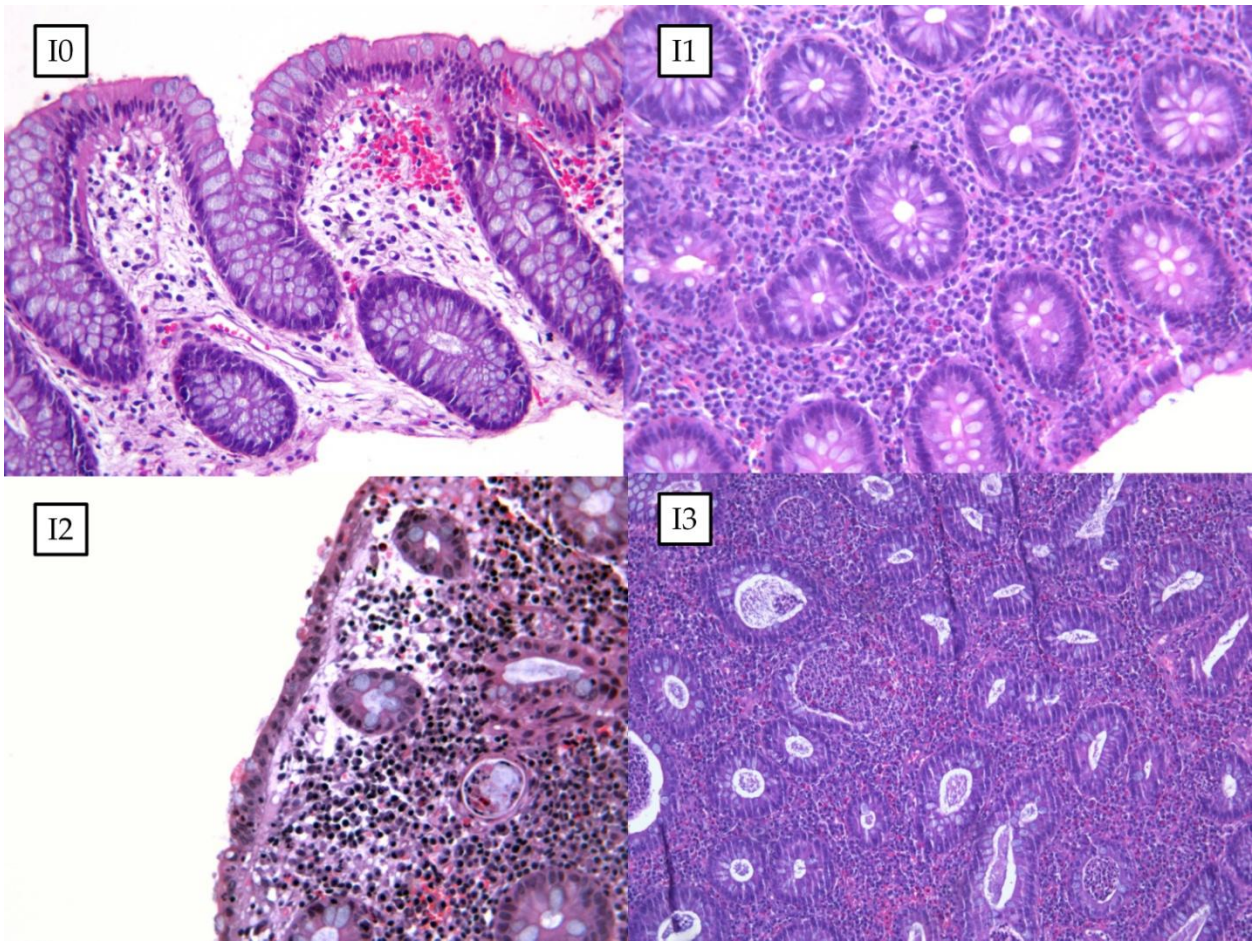
This score was applied for each biopsy site, for all children, for the first and the second colonoscopy, unaware of the grade previously given.

Picture 1 shows architectural score and Picture 2 inflammatory score.



Picture 1: haematoxylin eosin stains, 40x 100x and 200x magnifications.

A0: no architectural alterations: crypts are well oriented and mucin retained. A1: mild architectural alteration: focal crypt branching, some mucin depletion. A2: moderate architectural alteration: diffuse crypt distortions. A3: severe architectural alteration: crypt distortions, brunching, atrophy.



Picture 2: haematoxylin eosin stains, 100x and 200x magnifications.

I0: no inflammatory alterations: no increase of mononuclear cell of the lamina propria. I1: mild inflammatory alteration: increase of lymphocytes and plasma cells in the lamina propria. I2: moderate inflammatory alteration: activity is present. I3: severe inflammatory alteration: diffuse cryptitis and crypt abscesses, as well as ulcerations.

Hereafter, scores were compared between the first and the second colonoscopy, one year after the beginning of the Azathioprine therapy, as shown in picture 3.

Patient	Disease	Location	First biopsy architecture (A)	First biopsy inflammatory activity (I)	Second biopsy architecture (A)	Second biopsy inflammatory activity (I)
XY	CD	Ileum	0	0	0	0
		C. ascending	2	2	1	0
		C. transverse	2	3	0	1
		C. descending	0	0	0	0
		C. sigmoid	1	0	0	0
		C. rectum	0	0	0	0

Picture 3: example of score match after the second colonoscopy. Scores are matched site by site for both architecture and inflammation. In this case, at the second biopsy there are improvement for both architecture and inflammatory scores at the sites were the scores were altered (c. ascending and c. transverse).

At this point there were copious data to compare to produce MH.

To appreciate the difference between the first and the second biopsies, we defined three categories: improvement, worsening and no variation. Improvement was given when the score decreases from 2 or 3 to 0 or 1; worsening, for the opposite; no variation when the ranges remained between 0 and 1. When data did not change between 2 and 3, as no MH was reached, then it was interpreted together with worsening.

This applied for each biopsy site comparing the first and the second colonoscopy.

Successively, a comprehensive evaluation for each children was made, following criteria expressed in table 1.

Matching first biopsies and second biopsies:
When in all / nearly all intestinal segments scores improved:
Architectural improvement
Inflammatory improvement
Architectural and inflammatory improvement
When in all / nearly all intestinal segments scores worsened:
Architectural worsening
Inflammatory worsening
Architectural and inflammatory worsening
When data did not change significantly:
No variation
When data significantly changed from a segment to another:
Contrasting data

Table 1: synthesis of MH evaluation for all biopsy sites for each children at the end of the study.

Finally, MH ranged between 4 categories: improvement, either only architectural or only inflammatory or both improvement; worsening, either only architectural or only inflammatory or both worsening; no variation between first and second colonoscopies; contrasting data, when both improvement and worsening were present, either at the same site between architecture and inflammation, or between different colonic sites.

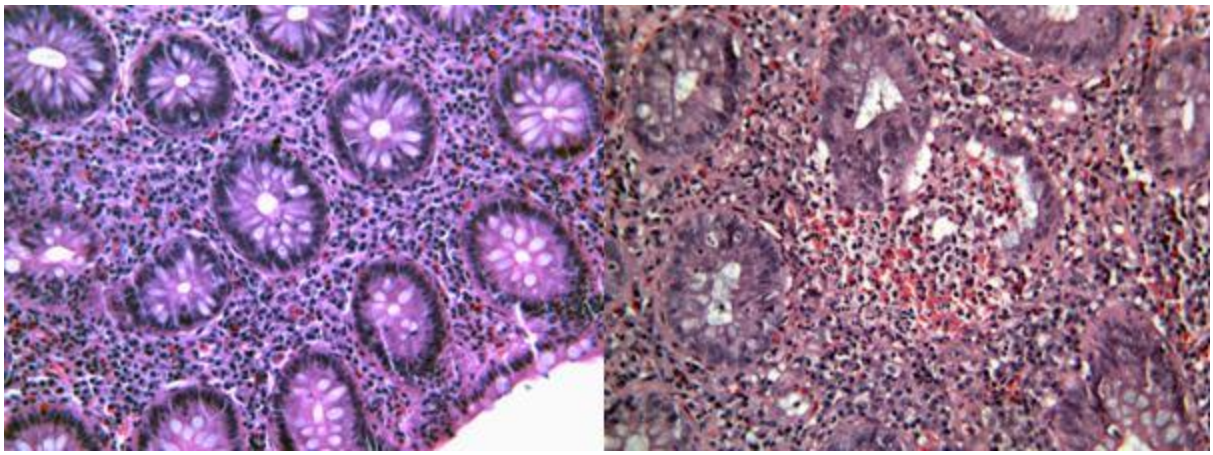
3.2.2 SECOND MH SCORE

In the second score, the activity score (AS), all biopsies were evaluated looking only for the presence of activity.

In this case the score had no grade and, independently of the site, when activity was present, the case was assessed positive for activity.

Virtually, activity was present in all cases at the first colonoscopy and, when absent at the second colonoscopy, they regressed, and when present, no matter of the biopsy site, it was persistent.

Picture 4 shows two examples of the score.



Picture 4: haematoxylin eosin, 200x magnification. Picture in the left shows colonic mucosa without signs of activity (regressed activity), while on the right crypt abscesses are evident (persistent activity).

3.2.3 IMMUNOHISTOCHEMISTRY ANALYSIS

After the first two evaluations, a biopsy site in which activity was present at the first colonoscopy together with corresponding site at the second colonoscopy were studied with immunohistochemistry for anti CD3 (Ventana CONFIRM anti-CD3 (2GV6)) CD4 (Ventana CONFIRM anti-CD4 (SP35)) CD8 (Ventana CONFIRM anti-CD8 (SP57)) CD20 (Ventana CONFIRM anti-CD20 (L26)) CD68 (Ventana CONFIRM anti-CD68 (KP-1)) and TNFα (N-19 Santa Cruz Biotechnology) to define the inflammatory cells composition.

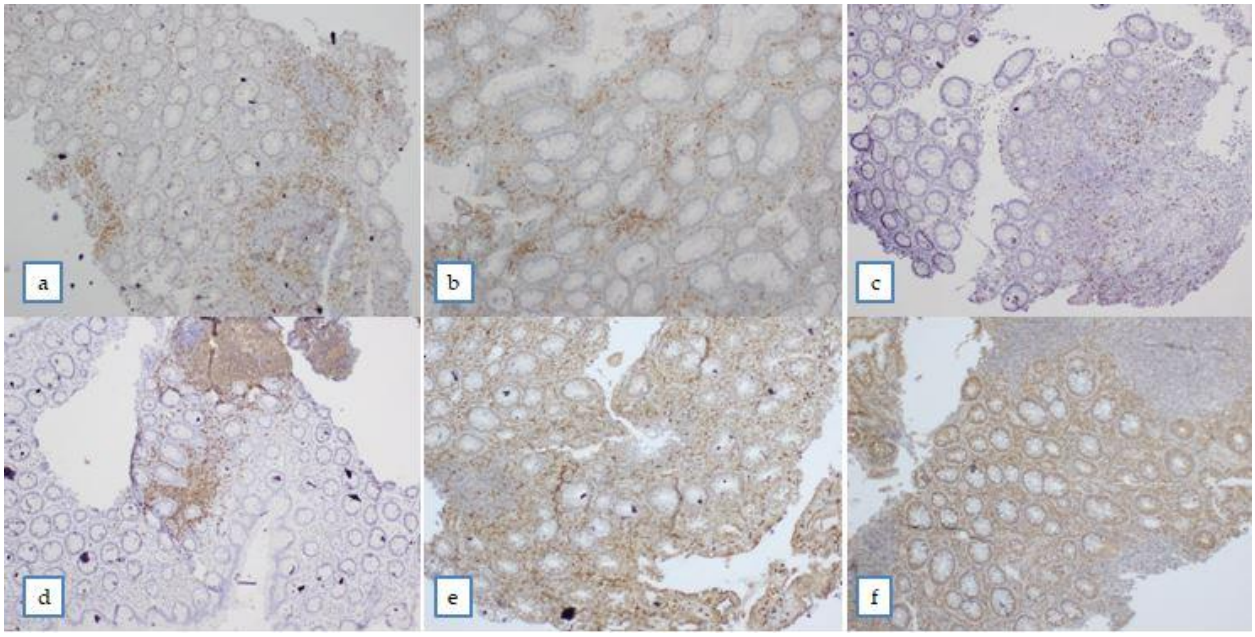
Immunohistochemistry analysis was performed with Ventana BenchMark XT Automated IHC/ISH slide staining system following producer protocols and dilutions.

Slides evaluation was expressed on percent scores for all antibodies except TNFα.

The positivity of TNFα was considered only in the inflammatory cell background and was simply divided in low expression and high expression.

A mean has been produced for all biopsies separating first from second biopsies.

Examples are given in picture 5.



Picture 5: immunohistochemistry, 100x magnification. Antibodies anti CD3 (a), CD4 (b), CD8 (c), CD20 (d) and CD68 (e) were evaluated as percent score of the whole inflammatory infiltrate. Antibody anti TNFα (f) in picture was evaluated high.

3.3 ENDOSCOPY AND PATIENTS FOLLOW UP

Endoscopy MH was procured from pediatrics of University Federico II and produced using the Mayo endoscopic sub-score and the Simplified Endoscopic Score for Crohn's disease.

Patients follow up was intended as the availability of further biopsy samples at our Institution up to 24 months after the second colonoscopy. Fifteen cases of CD and 16 cases of UC had follow up.

3.4 STATISTICAL ANALYSIS

Data presented in this work are given in whole numbers and percentages rounded to the nearest whole numbers.

Data obtained from the evaluation of the two histological MH scores were compared to Endoscopy scores. MH data were also compared to follow up data.

Statistical significance was tested, when appropriate, with Pearson's chi-square test settling statistical significance at $p<0.10$.

4. RESULTS

4.1 FIRST MUCOSAL HEALING SCORE, (CAIS)

With this score each patient had four possible MH score: improvement, worsening, no variation and contrasting data. Table 2 summarizes results obtained. With this score, the definition of MH is an improvement of architectural, inflammation or both in all or nearly all biopsies after the therapy. In this experiences, MH was reached in 27 cases (53% of patients). Among them, 13 cases of CD (54% of patients) and 14 cases of UC (52% of patients). In 7 cases (14% of patients) MH was not reached (5 CD and 2 UC). Three cases (6% of patients) had no variation (2 CD and 1 UC) and were not considered as improvement.

The most problematic consideration for this score is the high number of cases with contrasting data: 14 cases (27% of patients, 4 CD and 10 UC). This happened in all cases in which either the result of a biopsy site was totally different from another, or when at the same site architecture and inflammation were totally opposed.

	CD (24)	UC (27)
Both architectural and inflammatory improvement	8	8
Architectural improvement	0	1
Inflammatory improvement	5	5
No variation	2	1
Architectural worsening	0	0
Inflammatory worsening	2	0
Both architectural and inflammatory worsening	3	2
Contrasting data	4	10

Table 2: Results of MH CAIS separating CD and UC.

4.2 SECOND MUCOSAL HEALING SCORE, (AS)

The score has been produced comparing the first with the second colonoscopy at the end of the therapy. Table 3 summaries results obtained. MH was considered reached when no activity was present at the second colonoscopy. MH was reached in 35 cases (68% of patients): 16 CD and 19 UC (respectively 66% and 70% of cases).

	CD (24)	UC (27)
Regressed Activity	16	19
Persistent Activity	8	8

Table 3: Results of MH AS separating CD and UC.

4.3 IMMUNOHISTOCHEMICAL ANALYSIS

Antibodies expression is illustrated in table 4.

Results are given in percent scores of inflammatory infiltrate cells as a mean rounded up with 10% intervals, except for TNFα where results ranged from low to high.

There is generally a small meaningful change in the infiltrate between first and second biopsy after the therapy. In CD, we have observed a decrease in the infiltrate of T lymphocytes CD3 and CD8; In both CD and UC we have noticed a decrease of the expression of TNFα.

	CD (24)		UC (27)	
	First biopsy	Second biopsy	First biopsy	Second biopsy
CD3	30%	20%	20%	20%
CD4	30%	30%	30%	30%
CD8	20%	10%	10%	10%
CD20	20%	20%	20%	20%
CD68	30%	30%	30%	30%
TNFα	high	low	high	Low

Table 4: Percentages of antibodies expression differentiated between CD and UC and between first and second biopsy. TNFα is given as a mean between high and low expression.

4.4 ENDOSCOPY AND PATIENTS FOLLOW UP

Endoscopy MH was reached in 36 children (71% of cases), 15 patients affected by CD (63%) and 21 affected by UC (78%).

Thirty-one patients were re-biopsed between 12 and 24 months after this therapy: among them 11 patients had disease relapse (5 CD and 6 UC).

4.5 RESULTS CORRELATION

First comparison is between endoscopic MH and pathological MH, CAIS and AS.

Results are shown in table 5.

	CD (24)	UC (27)	IBD (51)
ENDOSCOPY	15 (63%)	21 (78%)	36 (71%)
CAIS	13 (54%)	14 (52%)	27 (53%)
AS	16 (66%)	19 (70%)	35 (68%)

Table 5: Comparison of MH reached by endoscopy and pathology (CAIS and AS). Results are shown in total numbers of patients and percentages of them.

When results were grouped for both IBD, correlation between AS and Endoscopy was statistically significant, while it was not statistically significant between CAIS and Endoscopy.

When results are separated between CD and UC, there is statistically significant divergence in predicting UC MH between the two pathological scores, while not in predicting CD MH.

The second comparison is between the pathological MH scores and the follow up.

Thirty-one patients had pathological follow up: among them 11 patients had disease relapse (5 CD and 6 UC) and 20 patients were in release phase (10 CD and 10 UC).

Agreement between MH and follow up was found in 17 patients for the CAIS and 20 patients for the AS (respectively 55% and 65% of cases).

Relapses were predicted in 3 cases by CAIS (2 CD and 1 UC) and 5 cases by AS (3 CD and 2 UC), (respectively 27% and 45% of cases).

Releases were predicted in 14 cases by CAIS (7 CD and 7 UC) and 15 cases by AS (8 CD and 7 UC), (respectively 70% and 75% of cases).

Both scores statistically correlate in prediction of releases and relapses.

Last comparison is between children younger then 10 years old.

This subpopulation of study was composed of 9 children, 4 MC and 5 UC. Among the 4 affected by MC, 3 had at MH CAIS improvement and 1 contrasting data, while at MH AS all had regression of activity. Three of them had pathological follow up and remission.

Among the 5 affected by UC, they all had at MH CAIS improvement, while at MH AS one of them had persistent activity. Two of them had pathological follow up, one remission and one relapse (he did not have persistent activity at MH AS).

5. DISCUSSION

In this work we have explored the role of pathologists in producing MH to the management of pediatric patients affected by IBD, both UC and CD.

At the beginning of our investigation no MH definition in pathology existed, and also the role of pathological MH for adult patients was not established (18).

The importance of the evaluation of MH comes from recent works that agree upon the importance of MH in the management of IBD patients: those who reach MH get a longer release free from disease (8).

So MH is now being considered as the endpoint of a therapy for clinical trials, and the evidence of MH is a better predictor of release then clinical and laboratory remission alone.

Patients not controlled with 5-ASA and steroids, that need to take harder drugs, are candidate to be controlled with MH together with clinical remission.

MH has been recently introduced in clinical trials and definition is limited to the drug used in the trial examined. This is the implication for which we have worked on patients that underwent all the same therapy, the immune-modulator drug Azathioprine.

Despite difficulties in finding a universally shared definition of MH in endoscopy, definitions varying between different studies generally gave satisfactory results on its evaluation (18).

In pathology, the situation was less explored and preceded by a generally inconclusiveness of pathological scoring systems for IBD (21).

Pathological features of UC made it the best IBD to be studied for pathological scoring systems.

Different scoring systems have been proposed: generally, evaluation of inflammatory activity and of architectural damages are included in these scores. The amount of activity is easier to produce. However, no agreement was found in the past and no definite usefulness was found in producing a degree of activity (20) .

One of the reasons for this failure is that pathologists express evaluations for each colonic site biopsied, while endoscopists give an overall definition of the disease, especially for UC.

However, pathology has two relevant qualities that incentivize the seek for pathological MH: first, activity in biopsies is still detectable when endoscopy is mute, making pathology the strictest index in the inflammation activity evaluation. Second, pathology can inform on the state of architectural damage of the mucosa, a valuable marker for chronicity and severity of the diseases.

So, starting from these considerations we proposed a pathological MH comprehensive of both values, defining pathological MH as a architectural and/or inflammatory improvement of the mucosa after the therapy.

And so we produced the first MH score proposed in this work, the combined architectural and inflammation score (CAIS).

With this score, both perspectives available to pathologists were investigated.

Our results were discouraging: correlation with endoscopy in reaching MH was low and not statistically significant when both IBD were grouped.

In a relevant percentage of patients (27%) we were not able to produce a MH evaluation.

When CD and UC patients were distinguished, results were superior for CD.

A promising result was produced comparing MH results to follow up available data, in which prediction of release was reached in 70% of patients (both IBD).

Our model had two interesting innovations: first, architectural damage and inflammation activity was studied and evaluated in a final inclusive score; second, each colonic site was compared at the beginning and at the end of the therapy.

On the contrary, this model was very hard to reproduce and difficult to perform.

The English literature proposed during the years of development of this research new works on this topic, and a new model has been designed, close to the endoscopy approach: the definition of MH as the absence of activity (24-25).

In this model, the contribution from the evaluation of architectural damage of the mucosa is not recognized.

MH is reached when no signs of activity are detectable. Like endoscopic MH does.

Following this model we developed our second MH model for pathology, the activity score (AS).

In this case, biopsies obtained at the second colonoscopy were simply screened for the presence of inflammatory activity: the presence of neutrophils through the epithelium. We did not contemplate if activity was absent where previously was present: if, at any site, activity was detectable, MH was not reached; if no activity signs were observable in any site, MH was reached.

This score was also easy to apply and to reproduce among pathologists.

By this definition MH compared with endoscopy was very similar.

Moreover, this was particularly evident when UC alone was considered (which is better investigated by endoscopy with biopsy then CD).

However, when only CD is considered, no statistically significant difference was given between the two pathological scores.

When both scores were compared with follow up data and analyzed for prediction of release, correlation between results was statistically significant.

However, prediction of release and relapse was lower by CAIS compared to the AS.

Pathological MH so defined, absence of activity, in our investigation, answered clinical need of MH definition and integrated well endoscopic MH.

Immunohistochemistry has been performed with the scope of examine in depth changes of the inflammatory cells involved in the IBD after Azathioprine therapy.

We found a generally decrease of inflammatory cells, but no significant change in the proportions of inflammatory infiltrate of the lamina propria. The only significant appreciable change was the decrease of T lymphocyte CD3+ CD8+ after the therapy in UC, expectable by a general remission of disease after the therapy.

Interestingly, we found a higher expression of TNFα in first biopsies and a significant decrease after the therapy.

Finally, we studied 51 patients in pediatric age, among them 9 children younger then 10 year old. We did not find any statistically relevant divergence in results between younger children and the other pediatric patients.

6. CONCLUSIONS

In this experience we analyzed a pediatric population of patients affected by IBD, both CD and UC, that underwent 1 year therapy with the immune-modulator drug Azathioprine.

We have correlated different definitions and scores of pathological MH to endoscopic MH and to pathological follow up.

The experience we had is that pathological MH, defined as absence of activity, and evaluated just looking for signs of activity at the second biopsies, with a complete mapping of colon including terminal ileum, is highly comparable to endoscopic MH, defined as absence of activity and evaluated with MAYO endoscopic scores.

This is particularly true when only UC is considered.

Moreover, this pathological MH is also significantly predictable of pathological disease release during follow up with endoscopy with biopsies.

Pathological MH, easily obtained evaluating mucosal biopsies collected during endoscopy, can be included in clinical management of pediatric patients.

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