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REVIEW ARTICLE

Results of the 2nd part Scientific Workshop of the ECCO (II): Measures and markers of prediction to achieve, detect, and monitor intestinal healing in Inflammatory Bowel Disease

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Abstract

The healing of the intestine is becoming an important objective in the management of inflammatory bowel diseases. It is associated with improved disease outcome. Therefore the assessment of this healing both in clinical studies and routine practice is a key issue. Endoscopy for the colon and terminal ileum and computerized tomography or magnetic resonance imaging for the small bowel are the most direct ways to evaluate intestinal healing. However, there are many unsolved questions about the definition and the precise assessment of intestinal healing using these endoscopic and imaging techniques. Furthermore, these are relatively invasive and expensive procedures that may be inadequate for regular patients' monitoring. Therefore, biomarkers such as

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C-reactive protein and fecal calprotectin have been proposed as surrogate markers for intestinal healing. Nevertheless, the sensitivity and specificity of these markers for the prediction of healing may be insufficient for routine practice. New stool, blood or intestinal biomarkers are currently studied and may improve our ability to monitor intestinal healing in the future.

Contents

1. Introduction

In February 2010 the SciCom launched its second pathogenesis workshop: "Relevance of Intestinal Healing for the Disease Course of IBD". In four different expert groups, the current knowledge about Mechanisms of Intestinal Healing (Basic science), Measures and Markers of Prediction to achieve, detect, and monitor Intestinal Healing, Impact of Intestinal Healing on the Course of IBD (Natural history), and Therapeutic Strategies to enhance Intestinal Healing (Therapy) was gathered and areas of future research identified. This manuscript summarized the results from the working group on Measures and Markers of Prediction to achieve, detect, and monitor Intestinal Healing, namely the assessment of Intestinal healing in inflammatory bowel disease using imaging techniques and biomarkers.

The assessment of tissue healing has become of paramount importance in inflammatory bowel disease. Simple clinical assessment does not reflect real activity and inflammation at the tissue level. Moreover, cumulative tissue damage generated by this persisting tissue inflammation leads to non reversible anatomic and functional consequences. Therefore, to evaluate both the natural history and impact of treatment in inflammatory bowel disease, standardized assessment of tissue healing must be available. To define the current situation and propose areas for future research, the working group of the second ECCO workshop on assessment of tissue healing in inflammatory bowel disease using imaging techniques and biomarkers, has addressed seven specific questions. These questions concern the definition of mucosal healing in Crohn's disease (CD) and ulcerative colitis (UC), the assessment of transmural and pathological healing in CD, the association with serologic and genetic markers, and the potential role of blood, stool and alternative biomarkers.

2. How to define mucosal healing in Crohn's disease?

Endoscopic activity may reliably be scored with one of the validated endoscopic activity scores, either the Crohn's disease endoscopic index of severity (CDEIS)[1] or the simple endoscopic score for Crohn's disease (SES-CD)[2]; both scores were shown to be highly reproducible (excellent inter-observer agreement was demonstrated) and they were prospectively validated.[1–3] Nonetheless both scores are rather complicated, therefore their use is restricted to clinical trials and is uncommon in routine clinical practice. Another very widely used score is the Rutgeerts' score for grading post-surgical recurrence severity. Rutgeerts's score was developed and validated[1,4,5] in order to predict a relevant difference in prognosis in the post-surgical setting. Although the score lacks formal evaluation of inter-observer agreement, it has been widely used across many different clinical trials and clinical series, and its prognostic value was

confirmed.[6–9] The main characteristics, strengths and weaknesses of these score are shown in Table 1.

With respect to endoscopic healing, it should be kept in mind that CDEIS[1] and SES-CD[2] were developed in order to transform the level of endoscopic activity of CD into a continuous variable, but not to predict relevant difference in prognosis, nor to determine cut-off values for endoscopic remission or for different levels of endoscopic activity. Their intrinsic complexity, together with the absence of validated score thresholds associated with specific prognostic values and with endoscopic healing, are the major weaknesses of these scores. Attempts to define endoscopic remission or minimal activity led to identification of CDEIS cut-off of lower than 3 points[10,11] and of SES-CD cut-off of lower than 5 points,[12] although in other studies the best prognosis seemed to be associated with a CDEIS or SES-CD scores of 0 points.[11,13] Currently available definitions of endoscopic healing that were associated with better clinical outcome (including less clinical relapses, surgical procedures and hospitalization) are thus multiple and heterogeneous: no mucosal ulceration observed in any of the 5 segments by endoscopy,[14] disappearance of all ulcerative lesions,[13,15] absence of mucosal ulceration,[16] CDEIS ≤ 2,[11] SES-CD ≤ 5, a Rutgeerts' score equal or lower than i1.[5–9,17,18] Variations in endoscopic scores were also used in post-hoc analyses of the EXTEND trial. A difference in prognosis for those patients attaining a more pronounced decrease in endoscopic activity was observed,[19] but they need further and prospective validation.

Although theoretically appealing, the prognostic relevance of histological healing has not been studied. Particularly, it is currently unknown whether histological healing can be achieved with current drugs in CD and what is its impact on disease outcome. Assessment and definition of mucosal healing in areas of the gastro-intestinal tract that cannot be reached by conventional endoscopy are also much less clearly documented. There are no published data on jejunal and proximal ileal lesion healing assessed with capsule endoscopy or with other device-assisted enteroscopy and there is no validated scoring system to assess small bowel endoscopic activity. However, current ECCO guidelines on small bowel endoscopy[20] report that small bowel capsule endoscopy has a potential role in the assessment of mucosal healing of lesions based on a small trial.[21] Other imaging techniques (ultrasound (US), magnetic resonance imaging (MRI)) may depict amelioration of the bowel wall pattern (thinner wall, better stratification, reduction of mesenteric fat proliferation, reduction in bowel wall enhancement, disappearance of edema) up to complete normalization of the bowel wall. Several serial MRI studies are available in the literature analyzing the pattern of fistula healing,[22–25] but no large quality data is available on the issue of luminal disease, especially with respect to response to medical treatment and prognostic impact,[26–30] and data on correlations between endoscopy and US/MRI are few.[31,32]

Key messages

- Endoscopic activity may be reliably scored in different clinical conditions:
  - Crohn's disease endoscopic index of severity (CDEIS) or simple endoscopic score for Crohn's disease (SES-CD) for luminal CD
  - Rutgeerts' score for anastomotic post-surgical recurrence of disease
- Composite indices are seldom used in clinical practice due to their complexity, while Rutgeerts' score use may be advocated routinely
- Endoscopic healing (EH) is commonly defined as the disappearance of all ulcerative lesions, although slightly different definitions were used in different studies

Table 1 Strengths and weaknesses of the more frequently used endoscopic diseases activity scores in IBD.

IBD score	Endoscopic variables	Strengths	Weaknesses
Endoscopic score for CD			
CDEIS	Deep ulceration, superficial ulceration, extent of ulcerated and affected surface, and narrowing	Gold standard for endoscopic assessment of CD, used in several clinical studies, reproducible	Complex for routine use in clinical practice. No well validated definition of mucosal healing
SES-CD	Size of ulcers, extent of ulcerated and affected surface, and narrowing	Simplified score for CD	No validated definition of mucosal healing
Rutgeerts score	Aphtoid lesions, inflammation, ulcers, nodules, and narrowing	Gold standard for the assessment of postoperative disease recurrence, validated cut-off value for the prediction of clinical relapse	Can be used only in operated patients for assessment of postoperative recurrence
Endoscopic score for UC			
Mayo endoscopic subscore	Erythema, vascular pattern, friability, bleeding, erosions, and ulcerations	Easy to use, 4-point scale (0–3). Mucosal healing defined by a score of 0 or 1	Not enough accurate, no discrimination between superficial and deep ulceration
Rachmilewitz endoscopic index	Granulation, vascular pattern, vulnerability of mucosa, and mucosal damage (mucus, fibrin, exudate, erosions, ulcer)	Used in many clinical studies, reproducible	No validated definition of mucosal healing
Modified Baron score	Friability, vascular pattern, bleeding, and ulceration	Easy to use, 5-point scale (0–4)	No discrimination between superficial and deep ulceration

- Degrees of endoscopic amelioration impacting on disease outcome were studied, and several thresholds in endoscopic scores were proposed (CDEIS = 0 / ≤ 3 / ≤ 6, SES-CD = 0 / ≤ 5)

Areas for future research

- Formal evaluation of inter-observer agreement for endoscopic scores of activity
- Larger trials with endoscopic healing as primary endpoint and relevant prognostic outcomes
- Development and validation of meaningful endoscopic response criteria with CDEIS/SES-CD as well as with more simple criteria for routine practice
- Optimal assessment of mucosal healing in the small bowel using small bowel capsule endoscopy and/or imaging techniques.

3. How to define mucosal healing in Ulcerative colitis?

Truelove and Witts were the first to report on the sigmoidoscopic appearance of the gut mucosa during a placebo-controlled trial of cortisone for the treatment of active disease. Endoscopic lesions were classified as normal or near normal (slight hyperemia or granularity as only abnormal finding), improved, or no change or worse.[33] Thereafter, several endoscopic scoring systems were developed using different items and definitions of mucosal healing. The Baron score[34] and the Powell-Tuck sigmoidoscopic assessment[35] focus on the assessment of severity of bleeding without considering ulcers or other mucosal changes. The Baron score, distinguishing three grades of activity, was commonly used to evaluate the degree of activity endoscopically. The most commonly used is the endoscopic component of the Mayo score.[36] Schroeder et al. performed serial flexible proctosigmoidoscopic assessments during a placebo-controlled trial of oral delayed release mesalamine for the treatment of UC. The appearance of the rectal mucosa was described using a 4-point scale (0–3) and was called the Mayo score.[36] Currently, the Mayo score is preferred in clinical studies. When using the Mayo endoscopic subscore, mucosal healing is defined as a score drop to Mayo score of 0 or 1 (Mayo 0 = normal or inactive disease and Mayo 1 = mild disease – erythema, decreased vascular pattern, mild friability). These criteria were used in several clinical trials.[37–43] However a more recent recommendation of the Food and Drug Administration is to consider any friability as non-healed mucosa. The Mayo score does not distinguish deep ulcers from superficial ulcers and is not useful for the evaluation of severe colitis. The score reproducibility was formally evaluated in a prospective study[44] which outlined that adequate reproducibility in the score application was achieved only for expert endoscopists. Rachmilewitz performed serial endoscopic assessments during a controlled comparison of coated mesalamine and sulfasalazine for the treatment of active UC.[45] In this trial, an instrument consisting of four items was described: granulation scattering reflected light, vascular pattern, vulnerability of mucosa, and mucosal damage (mucus, fibrin, exudates, erosions, and ulcer). Scores range from 0 to 12 points. Endoscopic remission was defined as an endoscopic index score of 0–4 points. The instrument has not yet been validated. Feagan et al.[46] performed serial endoscopic assessments during a placebo-controlled trial of anti-α4 β7 integrin antibody (MLN-02) for active UC, describing endoscopic activity on a 5-point scale (0–4). With this modified Baron Score, endoscopic remission was defined as a Score of 0. Endoscopic response was defined as an improvement of the Modified Baron Score of at least two grades from baseline. Neither the Modified Baron Score nor the definitions of endoscopic remission or endoscopic response have been validated. The reproducibility of scoring individual lesions and overall severity resulted good-to-excellent in a more recent study.[47] This study proposed a new, more detailed and complicated endoscopic score, named ulcerative colitis endoscopic index of severity (UCEIS), that has been formally validated but is only published in abstract format.[48] The strengths and weaknesses of the more frequently used endoscopic diseases activity scores in UC are presented in Table 1.

The risks of relapse or colectomy are important clinical outcomes, and seem to be reduced by attaining mucosal healing.[49,50] However studies specifically aimed at analyzing risk factors for disease relapse generally considered active endoscopic picture as an exclusion criterion. Nevertheless, a small trial published only as abstract[51] demonstrated that patients in clinical remission with endoscopic activity grade Mayo 2–3 had a substantially higher risk of clinical relapse as compared to those with endoscopic activity grade Mayo 0–1, after a course of mesalamine. More recently[52] it was shown that patients attaining clinical remission but with remaining signs of endoscopic activity (about one quarter of patients after a steroid course) had significantly higher risks of adverse outcomes and colectomy, with odd ratios ranging from 2 to more than 6 for different outcomes. The goal of reaching substantial endoscopic healing in ulcerative colitis is also supported by its protective effects on UC-related cancer incidence.[53] The same study supported an even greater importance of histological inflammation in the risk of colorectal cancer development in ulcerative colitis, with odd ratios between 4.7 and more than 6.

Key messages

- The most commonly used endoscopic activity score is the Mayo endoscopic score, with the commonly accepted criterion for endoscopic healing being Mayo grade 0 or 1(although any friability should be considered as non healed mucosa)
- Mucosal healing substantially reduces the risks of both clinical relapse and surgery
- There is a potential relationship between endoscopic healing and cancer risk reduction
- Histological healing appears to be preferable for cancer prevention, but limited data on its scoring are available
- Relevance of histological healing on disease recurrence is uncertain

Areas for future research

- Further evaluation of agreement for Mayo score and generation of more reproducible scoring systems
- Relevance and interpretation of heterogeneous severity of the endoscopic activity along the colon
- Definition and evaluation of prognostic value of histological healing in ulcerative colitis.

4. How to define transmural healing in Crohn's disease?

Eradication of inflammation in all the layers of the bowel wall would be a logical goal of treatment. It has been

suggested[54] that mucosal healing may be a minimal therapeutic goal in CD, as in the presence of a pathologic process characterized by transmural inflammation, healing of the most superficial layer only seems to be too little and too partial. There are, however, no published studies in which transmural healing was used as the major therapeutic endpoint of a CD patient treatment, while mucosal healing is becoming more and more a therapeutic goal.[10,13,15,16,55] Therefore any recommendation to use transmural healing as a treatment endpoint, although logical, is not evidence-based. In a recent study[56] comparing colonoscopy and PET-CT, only 35 out of 56 intestinal segments highlighted by ^{18}F-2-deoxyfluoroglucose (^{18}F-FDG) had detectable mucosal lesions at colonoscopy. Although these segments could have been a false positive for FDG uptake, the presence of wall thickening on CT and histological evidence of inflammation in some of these endoscopic negative segments support the possibility of mucosal or submucosal inflammation in the absence of endoscopic mucosal lesions. By contrast, another study observed that alterations in MRI findings were very rare in the presence of a normal mucosa at endoscopy.[30] In order for transmural healing to become an accepted endpoint of treatment, beneficial outcomes associated to transmural healing should be clearly demonstrated. Most CD patients have inflammatory disease at diagnosis and develop complicated disease patterns as the disease evolves.[57,58] A long term therapeutic goal should be aimed at prevention of this pattern of evolution. It would be of interest to determine the differential predicting accuracy of transmural healing and mucosal healing on disease progression. Both MRI and CT scan are very useful techniques to detect transmural inflammation and extraluminal complications. While the role of MRI and CT-enteroclysis or enterography for the study of the small bowel is well established in several studies, less is known about assessment of colonic transmural pathology.[30,59] Transabdominal US can also be used to detect bowel wall thickening, to aid disease diagnosis and complication assessment.[61–63] Its contribution to clinical decision-making and its prognostic role[60,64] are relevant, provided that an expert operator is available, as high inter-observer variation and lack of standardization were historically attributed as limitations to such technique.[32,65] CT, US, and MRI have consistently been shown to possess superior sensitivity and specificity compared to conventional small bowel follow-through (SBFT) for the detection of luminal lesions in proximal small bowel, providing additional invaluable information on the presence of extraluminal complications.[66–68] These facts, always keeping in mind risks associated to radiation exposure,[69–72] led to the general recommendation of using cross-sectional imaging modalities for the assessment of CD lesions in the more proximal intestine and for assessment of stricturing and penetrating complications. MRI or US should be used wherever possible to limit radiation exposure. Future research should be focused on standardization of the preparation, technological aspects (protocols), and imaging criteria used to assess CD lesions.

The use of labeled markers that reliably accumulate in pathologically hypermetabolic cells associated with inflammation is attractive for the diagnosis and follow-up assessment of patient progress in CD, particularly to assess transmural healing. It provides an added dimension to investigations based on structural changes associated with inflammation obtained from CT or MRI enterography and US.[32] Scintigraphy has not been widely adopted mainly because the most sensitive and specific method based on 99mTc-HMPAO (Tc-hexa methyl propylene amine oxine) labeled white blood cells requires extracorporeal labeling and more specific methods using labeled antibodies to cytokines have still not been sufficiently developed.[32,73–76] Nonetheless scintigraphy, due to its relatively low radiation exposure, was proposed as a useful technique in the diagnostic workup of pediatric population with suspected or known IBD.[77–79] Accumulation of ^{18}FDG in hypermetabolic cells has relevant potential for IBD diagnosis and monitoring, and may highlight active inflammation in CD when using analysis of positrons emitted during ^{18}F decay by means of positron emission tomography (PET). Combining PET with CT imaging enables localization of inflamed intestine with good spatial resolution.[80–82] A strategy whereby initial combined PET-CT imaging and PET alone as follow up to assess healing might have value[83] since the radiation dose from FDG is relatively low. The recent introduction of PET-MRI may be an elegant way of reducing radiation exposure and allow repeated assessment. However, it will probably take considerable time before this technique becomes generally available.

Key messages:

- Mucosal lesions should be considered as a standard for assessment of disease evolution and therapeutic efficacy, until the superiority of other measures of structural damage is shown
- Transmural healing in CD is a matter of growing interest, as the disease is not purely mucosal. However reliable definitions of transmural healing are not yet available.
- Many imaging techniques were validated for disease diagnosis and for detection of disease activity [magnetic resonance (MRI) or CT scanner entero-graphy/-clysis, bowel ultrasound (US), scintigraphy, positron emission tomography (PET)-CT, single-photon emission computed tomography (SPECT)], but none of these techniques was validated for definition of transmural healing
- Non irradiating techniques should be preferred, especially in young patients

Areas for future research

- Standardization of imaging techniques (US, MRI) and of alternative imaging (especially PET-CT/PET-MRI) with evaluation of their performance with major prognostic goals set as outcomes
- Standardized definition of transmural healing
- Added value of transmural healing over mucosal healing and its impact on long term evolution of CD.

5. How to assess fistulae and strictures in Crohn's disease (pathological healing)?

5.1. Stricturing disease

A high proportion of patients will develop strictures over the course of CD. This is probably part of the healing process in transmural inflammation, although very often, strictures contain both fibrotic and inflammatory components.

5.2. Definition of stricture

The most reliable definition of a stricture is a localized, persistent bowel narrowing whose functional effects may be judged from prestenotic dilatation.[84,85] However, various definitions of strictures have been used in radiological studies, including the presence of severe luminal narrowing in regions of bowel wall thickening with or without prestenotic dilatation.[86]

5.3. Radiological techniques for the detection of strictures

Optimal CT or MRI assessment of intestinal wall abnormalities requires luminal distension and systematic examination using nonabsorbable oral contrast. CT or MRI enterography is currently accepted as a preferable alternative to CT or MRI enteroclysis, being as accurate and better tolerated.[87] The accuracy of both CT and MRI enterography or enteroclysis for the detection of small bowel strictures is high.[26,88–90 26,91–94] US has also shown high diagnostic accuracy for the detection of small bowel strictures[61–63,86,95] but the sensitivity of US is lowest for those strictures located proximal small bowel segments.

5.4. Comparison between radiological techniques

Direct comparison of CT and MR for the diagnosis of a variety of small bowel lesions including IBD demonstrates similar high sensitivities and specificities for both techniques.[96] Studies that directly compared barium studies with MR enterography or enteroclysis found no differences in the detection of strictures but a more detailed characterization with MRI.[66,97,98] Strictures are potentially amenable to surgical treatment, and cross-sectional techniques allow better mapping and provide additional important information such as the identification of potential inflammatory masses or other associated complications (e.g., fistulas or abscesses) and the identification of their relationships with other structures. However, none of the aforementioned studies comparing radiological techniques included an accepted reference standard, and this is an important limitation.

5.5. Differentiation between fibrotic and inflammatory strictures

Theoretically, the presence of fibrotic obstruction favors surgical treatment, but the frequently present overlap between inflammation and fibrosis represents a difficulty for decision making on the best treatment option. Bowel thickening reflects both the presence of an inflammatory component and fibrosis in deep layers. Hyperenhancement and edema at CT and MRI may be more specific for inflammation. One prospective study correlated the wall echo pattern at US with histology after surgical resection. The US stratified echo pattern (different echogenicities in the different bowel layers) at the site of strictures may help to detect collagen deposition.[61] Fibrosis is usually seen as a hyperechogenic band, in contrast with other layers of the bowel, conferring the stratified echo pattern. No published study so far disclosed imaging parameters that can reliably predict the response to therapy in the setting of stricturing CD.

5.6. Penetrating disease

Penetrating CD, defined according to ECCO as the presence of internal fistulas and/or abscesses, should be identified by using diagnostic tools able to detect the mural and extramural complications of the disease. For intra-abdominal penetrating disease, CT/MRI enterography or enteroclysis and US are more sensitive than standard barium examinations. Likewise, the superiority of CT with respect to barium studies has been confirmed in all published papers. To date, no studies have specifically compared CT and MRI for imaging penetrating CD. Only one study has compared US and MRI for assessing extension and inflammatory activity in CD.[99] In this study, both techniques showed high diagnostic accuracy for the diagnosis of CD and its penetrating complications, with fair agreement between the two procedures.

The trials on the anti-TNF agents in fistulizing CD were mainly focused on perianal disease. The main endpoint was a greater than 50% reduction in the number of draining fistulas. The complete resolution of drainage was a secondary end-point. These end-points were determined using the Fistula Drainage Assessment Measure, which characterizes fistulas as open (i.e. actively draining) if an investigator can express purulent material from the fistula with the application of gentle pressure to the tract. However, several reports using either MRI or endoscopic ultrasound (EUS) to assess perianal fistulae, have shown the persistence of active fistulae even in absence of external drainage.[24,25,100] These findings were recently confirmed,[101] showing that only 28% of the 46% of CD patients considered in clinical remission with anti-TNFs showed the healing of perianal fistulous track at MRI. In concordance with the MRI findings, EUS showed complete healing of the fistula track in only one third of the patients with closure of external fistula.[102] Persistence of active fistula track at EUS examination[103] was observed in 48% patients, 86% of whom presented the complete cessation of drainage on the Fistula Drainage Assessment Measure. On the basis of these observations, "deep healing" of fistulas can be considered a relatively rare outcome. No study has compared the diagnostic accuracy of MRI and EUS in defining the activity of perianal fistulas in CD. MRI and EUS could be considered useful tools for guiding treatment decisions (e.g., stopping anti-TNF, removing setons, etc.). Fistula tracts that show initial improvement on MRI and/or EUS 12–14 weeks after treatment have a good chance of remaining healed. However, it is uncertain whether periodical imaging re-evaluation regardless of the symptoms has a potential impact on clinical management. On the basis of the most recent evidence, the Fistula Drainage Assessment Measure should be considered inadequate for defining fistula outcome during and after therapy.

Key messages:

- MRI, and to a lower extent CT, due to radiation exposure, are preferred techniques in order to assess the presence of strictures
- US is an adequate alternative when an expert operator is available

- No diagnostic tool is at present able to discriminate between inflammatory and fibrotic component of strictures with certainty
- MRI is the preferred technique for detecting penetrating complications of CD
- CT could be considered a suitable alternative only in case of limited accessibility to MRI or in the emergency setting
- Bowel US, provided an expert operator is available, is accurate and may serve as an alternative to other cross-sectional imaging techniques for penetrating CD
- No cross-sectional imaging technique was validated to-date for the assessment of internal fistulae and strictures healing in CD
- Tissue healing of perianal CD should be defined on the basis of MRI (or EUS) findings, although none of the techniques was evaluated for reproducibility

Areas for future research

- To explore the ability of cross-sectional imaging techniques to predict therapeutic responses in CD, in stricturing and fistulizing lesions
- To analyse the ability of cross-sectional imaging techniques to highlight post-treatment changes in strictures and fistulae predictive of different outcomes in the long-term follow-up.

6. Are serologic and genetic markers associated with tissue healing?

In recent years strong emphasis has been placed on identifying markers that can serve in facilitating diagnosis, disease differentiation and prognosis. Biomarkers can be divided into long-term markers (e.g., genetics and serology) associated with clinical phenotype, and short-term markers, associated with flares or tissue inflammation. As far as the association with tissue healing concerns, genetic markers, and probably also serologic markers, could be associated rather with a tendency to real healing or to pathological healing and subsequent complications (see above) than with the presence of tissue healing at a given time point.

6.1. Genetic markers

The three common NOD2/CARD15 mutations were associated with ileal disease and fibrostenosing behavior, suggesting an inclination toward pathological healing.[104,105] In a recent study,[106] a more complex genetic model was used including both novel (e.g. ATG16L1, IL23R) and earlier (NOD2, IBD5 and DLG5) genetic markers. In this study, patients with CD with a more severe disease course, surgeries or an age of onset below 40 years had more risk-alleles compared to non-stricturing, non-penetrating behavior, no operations or age at onset greater than 40 years, again suggesting an association between these genetic variants and an inclination towards an absence of tissue healing or a pathological healing. However, the association became insignificant in longer disease duration, confirming that many other factors (e.g. clinical variables, disease phenotype at diagnosis, medical therapy) contribute to the long term evolution of disease phenotype. In an even more comprehensive study of clinical, demographic, serological and genetic factors associated with the development of complications, it was shown that homozygosity for the rs1363670 G-allele in a gene encoding a hypothetical protein near the IL12B gene was independently associated with stricturing disease behavior and with shorter time to strictures, especially in patients with ileal involvement.[107]

6.2. Serologic markers

Serologic markers include ASCA, gASCA ALCA ACCA AMCA anti-L and anti-C (the novel anti-glycan antibodies). Other microorganism-directed antibodies include OmpC, CBir1, I2 and APLA.[108] These may serve as tools for diagnosis, disease stratification and prediction of disease phenotype or progression. However, they are not able to predict tissue healing or rapid changes in mucosal status. While levels of serologic markers such as ASCA and the anti-glycan antibodies may slightly fluctuate over time, it has been reported that the dichotomous status of the markers (positive or negative for a respective antibody) in IBD patients appears to be widely stable in serial measurements in individual patients. Between 74.2 and 89% of the CD patients and 83.7 to 97.8% of the UC patients remained in the same status they had at the initial sample procurement.[109,110] A large body of information is available about seroreactivity to anti-glycan markers and complicated disease behavior in CD.[108,111–114] Most information is derived from cross-sectional studies with samples taken at various random points during the disease course, combining serum taken before, at the time of and after complications occurred. These studies first performed with ASCA,[115–117] then with single glycan markers,[110–113] including more recently anti-L and anti-C antibodies,[114] suggest an association between these markers and stricturing and/or internal penetrating disease. To add clinical value for these markers and really see whether they can predict pathological healing, one has to go beyond association studies and evaluate a predictive capacity of serum markers in respect to the occurrence of complicated CD-behavior and surgery. Limited information is available, mainly from pediatric cohorts indicating an increased likelihood for complicated disease courses with an increasing immune response to microbial components. In a retrospective pediatric onset CD cohort it was shown that ASCA can serve as a predictor for the earlier occurrence of complicated CD-behavior or surgery.[118] The largest study to date using a prospective study design indicates that an increasing immune reactivity to ASCA, anti-cBir1 and anti-OmpC indicated a faster progression towards complicated disease and surgery.[119] To date only one study is available assessing the predictive use of anti-glycan antibodies in adult CD[110]: in Fifty percent of cases sera were obtained within one year of diagnosis. Positivity for the single markers ASCA, ACCA, AMCA and anti-L, as well as an increasing number of positive markers out of the whole panel independently indicated faster progression towards a more complicated CD course, defined as the occurrence of fistulas, strictures or CD-related surgery. Even though this study was underpowered, it nevertheless suggests a predictive value of the markers for the natural history of CD, particularly a trend toward pathological healing. Disease location seems to act as an important confounder: serologic markers may not be sensitive enough to be used in patients with isolated colonic disease, since ASCA and gASCA, as well as several of the other glycan markers are associated with ileal and small bowel

disease location, and therefore negatively associated with isolated colonic disease. Prospective studies with a complex model including detailed disease phenotype and serology profile are needed to analyze the complex effect of serology on disease progression and long term tissue healing. In contrast, over the short term, positivity for ASCA does not seem to predict response, including tissue healing, to anti-TNF or 5-ASA therapies.[120,121] Finally, there is evidence from other gastrointestinal diseases that the change in serologic response may occur slowly. Most probably that is why only little variation was reported in antibody titres in IBD. Strong marker expression was observed for both ASCA[122] and the newly discovered glycan markers[123] in celiac disease at the time of diagnosis (ASCA 40–60%; any glycan marker positive in 66%), especially in patients with severe malabsorption (any glycans: 78%). However, the response was completely lost after long-term (2–2.5 years) gluten-free diet. This observation suggests that serologic markers might also change and become negative in subgroups of IBD patients who achieve long term complete mucosal healing, although such an hypothesis will require prospective studies to be assessed.

Key messages:

- Genetic markers are associated with the development of CD complications and may possibly allow in the future prediction of inability to heal or occurrence of pathological healing.
- Serologic markers do not fluctuate much and are not associated with tissue healing over the short term, but they could also predict inability to heal or occurrence of pathological healing.

Areas for future research:

- Confirm the predictive value of genetic and/or serologic markers on the ability to heal and the pattern of tissue healing in IBD.
- Assess the capacity to decrease the risk of development of complications in genetically or serologically-defined high risk patients by various therapeutic strategies.

7. What is the role of stool markers in the assessment of mucosal healing?

While a rather large number of studies have assessed the correlation between fecal calprotectin and endoscopic activity in CD, only few have tried to establish thresholds for the prediction of mucosal healing and/or non significant endoscopic lesions. In UC, even less studies have been performed, probably because a simple sigmoidoscopy can sufficiently provide an assessment of the situation for decision making. Globally, four studies are available in CD,[11,124–126] two in mixed populations of CD and UC,[127,128] and two in UC.[129,130] In CD, only one of these studies included a formal assessment of various thresholds for calprotectin to determine the optimal cut-off for the prediction of mucosal healing or non significant endoscopic activity.[11] In a first CD study, an abnormal value of calprotectin ($>$ 50 μg/g) was associated with the presence of endoscopically active disease (SES-CD$>$7).[124] In another study, fecal calprotectin$>$200 μg/g could predict endoscopically active disease (CDEIS$\geq$3) with a sensitivity of 70% and a specificity of 92%.[126] In a third study, at a threshold of 70 μg/g, the global accuracy for the prediction of endoscopically active disease was 87%.[125] In the fourth study (only published in an abstract form), performed on 85 patients, several thresholds of calprotectin were tested.[11] The optimal threshold to detect endoscopically inactive disease (CDEIS$<$3) was 250 μg/g with a sensitivity of 82% and specificity of 53%. In this study, combining hsCRP$<$5 mg/l together with fecal calprotectin improved the specificity to 72% while only slightly decreasing sensitivity to 74%.

In UC, only 2 studies have evaluated the ability of fecal calprotectin to predict mucosal healing.[129,130] In one of these, the overall accuracy for the detection of endoscopically active disease (Rachmilewitz score $\geq$4) was 89%.[129] This was superior to blood biomarkers (CRP, white cells count) and clinical activity index. In a pediatric study mixing 26 CD and 32 UC, PPV and NPV for the prediction of endoscopically active disease were 81% and 87% respectively.[127] In a third study, the overall accuracy of fecal calprotectin for the prediction of endoscopically active disease was around 80% both in UC and CD.[128] There is currently no published evidence-based answer whether calprotectin has an added value over endoscopic healing to predict disease outcome. However, the GETAID STORI trial (only published in abstract form) suggests that there may be an added value.[11] In this study, approximately 30% of the patients with full mucosal healing experienced a relapse of CD within one year after infliximab discontinuation. By multivariate analysis, fecal calprotectin level as well as high sensitivity CRP (hs-CRP) were found complementary to endoscopic activity score and the combination of these markers, together with some clinical characteristics allowed the identification of a subgroup of patients with a very low risk of relapse (around 10% over one year).

As far as the type of fecal marker, there is no evidence-based answer. Fecal calprotectin has been the most widely studied. Fecal lactoferrin seems to be generally as good as calprotectin to predict endoscopic activity of the disease[124,126,131,132] and might be better than calprotectin for assessing ileal disease activity.[133] S100A12 is another member of S100 family in addition to calprotectin (S100A8/S100A9). It has been studied only in a small series of patients with IBD and has been suggested to be more sensitive than fecal calprotectin[134] but this needs further validation. No studies have correlated S100A12 with endoscopic activity of CD or UC. Day-to-day variation of fecal markers has been inadequately studied.[135] As far as the optimal threshold of these fecal markers, there is currently no evidence-based answer either. However, it is obvious from studies correlating calprotectin to endoscopic activity scores, that no universal threshold can have a 100% accuracy. Particularly, approximately 15–20% of patients in full mucosal healing still have elevated fecal calprotectin levels, while approximately the same proportions of patients with significant endoscopic lesions have calprotectin level within the normal range.[126] What we currently do not know is if these "out of range" values reflect a particular stable biological trait in those patients and hence could be used as an individual threshold. Longitudinal measures and clinical follow up are needed to answer this important question.

Key messages:

- Calprotectin and other stool markers significantly correlate with endoscopic scores of activity and may become in the near future an alternative or surrogate marker of mucosal healing, at least for Crohn's colitis and ulcerative colitis.

- Optimal thresholds and optimal markers for assessing mucosal healing still needs confirmation from large prospective studies.

Areas for future research:

- Comparisons of various fecal markers and longitudinal follow up of stool markers aiming at clarifying whether clinically meaningful thresholds can be identified.
- Optimal stool markers for different disease locations and behaviors should be studied.

8. What is the role of blood markers in the assessment of mucosal healing?

Very few studies have assessed the correlation between blood markers and objective measurement of disease activity in the gut in inflammatory bowel disease. The marker which has been most broadly studied is CRP, and in the few studies where CRP was compared to other blood markers, such as inflammatory cytokines, cytokine receptors or other acute phase reactants, those were usually not found consistently superior.[3,108,124] In CD, circulating CRP levels were not found to correlate closely with endoscopic activity.[136] Particularly, the correlation between endoscopic index of severity (CDEIS) and CRP resulted statistically significant but very weak.[3,108,127] Still, an elevated CRP may increase the likelihood of presenting endoscopic lesions.[137] In UC, blood markers are less useful except for extensive disease[138] and fecal markers seem to be more appropriate for prediction of endoscopic activity [139]. This was confirmed in a recent Japanese study where the association between CRP and endoscopic activity was significant only for extensive but not for distal UC.[138] In a large transversal study including 164 CD patients, hs-CRP and IL-6 blood concentrations showed a similar correlation with endoscopic score of activity. The areas under the ROC-curve with these markers were between 70 and 80%.[124] No systematic search for optimal threshold of CRP in predicting mucosal healing in CD has been performed yet. In a recent study, the sensitivity and specificity of hs-CRP$<$5 mg/l to predict the absence of significant endoscopic activity of the disease (CDEIS$<$3) were 78% and 39%, respectively. These values were lower than for fecal calprotectin, but the combination of these 2 markers gave the best results.[11] The proposal to use hs-CRP came from the cardiovascular research in order to predict evolution in coronary heart disease. Since in cardiovascular disease normal methods for CRP measurement are not sensitive enough, a hs-CRP assay has been developed and is currently in use helping to predict the first or recurrent coronary events.[140] At this moment there is no evidence that measuring hs-CRP yields better results than conventional CRP measurement in IBD patients. Moreover another cheaper assay, the wide range CRP, measures concentrations ranging from 0.05 to 160 mg/l and yields results that correlate closely with those of hs-CRP detection kits. CD is usually a transmural disease, therefore it is probable that beside fecal markers, blood markers will be necessary to better reflect the whole pathology process. Here again, a correlation with transmural inflammation has been essentially shown with CRP. In a large retrospective study correlating endoscopic appearance of the terminal ileum, CT enterography features and CRP, a significant correlation was found between CRP and perienteric inflammation (increased fat density) but not with the intensity of the inflammation limited to the small bowel wall.[141] Regarding newer blood markers such as procalcitonin, phagocyte-specific S100 proteins, neutrophil gelatinase-associated lipocalin (NGAL) or ghrelin, no information is available concerning their correlation to mucosal or tissue inflammation. As we know from clinical practice and from clinical studies, an important percentage of patients with IBD do have normal indices of inflammation even in the presence of active disease. In order to identify markers relevant for each individual patient, it may be helpful in the future to assess a panel of potential markers to select, for each patient, the one which is best correlated with mucosal and tissue inflammation.

Key messages:

- In Crohn's disease, CRP may be a weak surrogate marker of mucosal healing.
- In extensive ulcerative colitis, a significant correlation between endoscopic disease activity and CRP values was shown.
- At this moment there is no evidence that measuring high sensitivity CRP yields better results than conventional CRP in IBD patients, included in the prediction of mucosal healing.

Areas for future research:

- Prospective analysis of correlations between CRP drop and mucosal healing.
- Study of new blood markers better correlated to gut inflammation.
- Analysis of the clinical impact of serial measurements of combination of blood markers.

9. Are there alternative biomarkers of interest?

Tissue factors mediating the inflammation in IBD are potential biomarkers for both prediction and monitoring of the clinical course of IBD and of response to therapy, including tissue healing. From a theoretical point of view, mucosal markers should be better predictors than serological, fecal or urine markers as long as the main inflammatory events are located in the inflamed mucosa. However, up to now no mucosal marker has been significantly associated with mucosal healing or to disease severity. For the large majority of the possible analyses, the rigorous sampling procedure required is not suited for daily clinical activity. Of the few exceptions are the samples for quantitative gene expression analyses using transport medium at room temperature for at least one week without degradation of RNA.[142] Nevertheless, the introduction of new mucosal markers for clinical use represents a great logistic challenge from biopsy sampling to the final step of analyses.

There are no reports which demonstrate that mucosal expression of TNF-α predicts clinical course and only few reports exist regarding the predictive value of mucosal TNF-α on response to treatment. Pretreatment mucosal TNF-α was negatively correlated to response to infliximab in CD[143] and in UC.[144] This disagrees with another report where low expressions of TNF-α predicted poor response to therapy in CD.([145]) Higher levels of TNF-α were also observed in corticosteroid non-responders than in responders to steroids

in UC[146] but this finding was not confirmed in another study.[147] Of interest is the microarray/RT-PCR study of Arijs and colleagues in which gene expression associated with non response to infliximab in UC was tested.[148] TNF-receptor (SF11B) predicted the clinical response. Other predictors were the glycoprotein STC1 maintaining the endothelial permeability, the tissue injury factor cyclooxygenase 2b molecule PTGS2 linked to the biosynthesis of prostaglandin, and finally the receptor for the proinflammatory cytokine interleukin-13 (IL-13-R) and the anti-inflammatory cytokine IL-11.

At present it is merely an optimistic thought, although logically attractive, that integration of serologic and stool markers with mucosal markers may increase reliability of the prediction of mucosal healing. New metabolomics techniques such as NMR spectroscopy of fecal extracts have demonstrated differences in metabolic profiles in IBD compared to healthy controls.[149] Moreover, in a mapping analyses of urinary metalloproteinases (MMPs) performed by Manfredi et al., MMP-2 and MMP-9/NGAL were independent predictors of CD and UC.[150] This opens the possibility for new non-invasive fingerprinting methods but its potential as a prognostic biomarker is unknown.[149] Moreover, urine markers represent a promising tool as the collection of urine samples is easy to perform and non-invasive. The parameters are mostly expressed as ratio to creatinine and therefore the more complicated sampling of 24 h urines is unnecessary.

Key messages:

- Molecular analysis of tissue samples is a promising approach, but problems with logistics, costs and technical complexity limit at present its current use.
- Urinary markers, chemokines, cytokines, cellular mediators and adhesion molecules may represent interesting candidates as alternative biomarkers.
- At present no alternative biomarker was formally tested for the evaluation of tissue healing and therefore such techniques are still not suitable for use outside of clinical trials.
- At present the only marker with some clinical relevance seems to be TNF-α expression, although its clinical relevance is still limited.

Areas for future research:

- Prospective studies with a broad fingerprinting assay from mucosa and body fluids aiming at identification of correlations with mucosal pattern and/or clinical outcomes.

Conflict of interest

MD received consultancy fees from Schering Plough, Abbott, MSD; received lecture fees from Schering Plough, Abbott, MSD, Sofar, Chiesi, Ferring, Nycomed. ID received consultancy fees from Abbott Laboratories, Merck, Bioline, Centocor; research support from Teva, Israel; lecture fees from Ferring, Schering Plough. MF received consultancy fees from Abbott, MSD, Bayer and Roche; received research grants from MSD, Roche; received lecture fees from Abbott, MSD, Bayer and Roche. GF received consultancy fees from Abbott Laboratories-Israel. WF received lecture fees from Schering-Plough, Abbott, Nycomed; research or educational grants from Schering-Plough, Abbott, Giuliani. PLL received consultancy fees from Schering Plough-MSD, Abbott; received research or educational grants from Schering Plough-MSD, Abbott, Astellas; received lecture fees from Schering Plough-MSD, Abbott, Ferring. JP received consultancy fees from Schering Plough, Abbott, MSD, UCB; received research or educational grants from, Schering Plough, Abbott; received lecture fees from Abbott, Ferring, MSD, and Schering Plough. JR received research grants from Abbott. EL received consultancy fees from Schering Plough, Abbott, MSD, Ferring, Shire, Millenium, UCB; received research or educational grants from MSD, Schering Plough, Astra Zeneca, Abbott; received lecture fees from Abbott, Astra Zeneca, Ferring, MSD, Schering Plough, Falk, Menarini, Chiesi, Nycomed.

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- EL and MD organized the workshop, on behalf of the ECCO scientific committee, drafted and edited the manuscript.
- All the authors wrote specific parts of the manuscript.
- All authors read and approved the final manuscript.

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