Video Article

Flexible Colonoscopy in Mice to Evaluate the Severity of Colitis and Colorectal Tumors Using a Validated Endoscopic Scoring System

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Abstract

The use of modern endoscopy for research purposes has greatly facilitated our understanding of gastrointestinal pathologies. In particular, experimental endoscopy has been highly useful for studies that require repeated assessments in a single laboratory animal, such as those evaluating mechanisms of chronic inflammatory bowel disease and the progression of colorectal cancer. However, the methods used across studies are highly variable. At least three endoscopic scoring systems have been published for murine colitis and published protocols for the assessment of colorectal tumors fail to address the presence of concomitant colonic inflammation. This study develops and validates a reproducible endoscopic scoring system that integrates evaluation of both inflammation and tumors simultaneously. This novel scoring system has three major components: 1) assessment of the extent and severity of colorectal inflammation (based on perianal findings, transparency of the wall, mucosal bleeding, and focal lesions), 2) quantitative recording of tumor lesions (grid map and bar graph), and 3) numerical sorting of clinical cases by their pathological and research relevance based on decimal units with assigned categories of observed lesions and endoscopic complications (decimal identifiers). The video and manuscript presented herein were prepared, following IACUC-approved protocols, to allow investigators to score their own experimental mice using a well-validated and highly reproducible endoscopic methodology, with the system option to differentiate distal from proximal endoscopic colitis (D-PECS).

Video Link

The video component of this article can be found at https://www.jove.com/video/50843/

Introduction

Murine endoscopy has been performed as a useful research tool for over a decade[1-3]. To date, most studies employing murine endoscopy have used rigid endoscopes, although some have also used flexible sigmoidoscopy. Murine endoscopy provides immediate results and a more objective estimate of the extent of intestinal normalcy, severity of inflammation, and tumor progression compared to indirect measures, such as body weight loss, diarrhea, and histology (which is suboptimal when lesions are patchy), while offering insights on the overall health of the colon. Most notably, endoscopy allows for the repeated assessment of laboratory animal models over time, as opposed to traditional histological examination that requires the animal to be euthanized and colons to be harvested for analysis[1]. Despite these advantages, the use of murine endoscopic technology has not been widespread and is limited by the lack of standardized protocols for implementation and scoring of pathological findings. Properly implemented, murine endoscopy holds great promise to further facilitate our understanding and characterization of animal models of multiple chronic gastrointestinal disease states, including inflammatory bowel diseases, colitis, and colorectal tumors.

The utility of murine endoscopic technology hinges upon its reproducibility and objectivity, which requires the existence of standardized examination procedures and a consistent, non-redundant, and reliable scoring system for intestinal pathologies. At least three descriptive scoring systems for endoscopic assessment of colitis[4-6], as well as for colonic tumors[4,7,8] in mice have been published. However, these reported approaches and scoring systems are not readily comparable across studies. In many cases, there is no clear definition of the criteria used for classification of lesions, and when criteria are given, they vary widely. Moreover, there are no reported scoring systems that integrate the assessment of both colitis and tumors, two of the most common colonic pathologies that can occur simultaneously and have interactive effects on outcome, into a single measurement tool. Finally, the endoscopic scoring systems that do exist for colitis often have inflammatory categories with limited discrimination (*i.e.* narrow scoring options over short integer scales, often 1-4) to properly represent disease progression scenarios and enable the use of parametric statistical analysis.

In this paper, we describe the use of a flexible endoscope to assess the severity of murine colitis and colonic tumors and describe a standardized protocol for employing this technology in the anatomical assessment of the perianal region, rectum, and distal colon. We illustrate effective troubleshooting during endoscopy to minimize trauma and image artifacts, and we describe a reproducible scoring system based on validated published clinical parameters. The scoring system integrates evaluation of both intestinal inflammation and tumors using a high level of discrimination with 12 possible grades of inflammation, tumor mapping and plotting options, and a novel decimal unit system (i.e., decimal identifiers) to highlight findings with specific diagnostic value (*i.e.* tumor development, complications during endoscopy, *etc.*). The use of decimal identifiers allows for rapid tracking of relevant data sets for further downstream analysis. Finally, we perform reliability and validity testing of the scoring system in multiple murine models of colitis and colitis-associated cancer (dextran sulfate sodium (DSS) induced-colitis, *Clostridium difficile* infection, and azoxymethane/DSS-induced colonic cancer).

Protocol

1. Setting up the Endoscopy System

1. Set up the endoscopy system before anesthetizing the mice, following the manufacturer's instructions. Use a video ureteroscope designed for humans for this protocol (**Figure 1**). The outer diameter of the insertion tube is 9.9 Fr (3.3 mm), its distal end is 8.5 Fr (2.8 mm), and the inner diameter of the instrumentation channel is 6 Fr (1.2 mm).
2. Although the use of endoscopes have not been linked to disease transmission [9], sterilize the endoscope and lens with 70% ethanol[10] or with appropriate techniques when working with spore-forming microorganisms. Prepare bacteriostatic sterile lubricant and non-scratching wipe tissues to saturate the endoscope tip.
3. Attach a 3 ml syringe full of air (or a suitable CO_2 gas mixture) to the top of the instrumentation channel.
4. Turn on the power, and set up the white balance in normal white light, as well as narrow band imaging (NBI) mode, when applicable. Since 2001, NBI has provided diagnostic imaging benefits as it emphasizes fine blood vessel structures on mucous membranes, including ulcerative and tumorous lesions, which have characteristic abnormal vascularization[11]. NBI-filter mode restricts the wavelength of captured light to 390-445 nm and 530-550 nm, which highlights structures with hemoglobin.
5. Print the assessment scoring form (**Figure 2**) to record your findings. Become familiar with expected normal and NBI images (**Figure 3A**) and the definition and grades of each criterion before the exam (see Section 6.1).
6. Prepare a colorant-based solution on phosphate-buffered saline (PBS) for chromoendoscopy, if desired. In chromoendoscopy, the exam is performed using a dye as contrast medium to highlight mucosal changes. Use methylene blue (0.1-1%) and indigo carmine (0.1%) solutions (**Figure 3B**). Note that methylene blue is an oxidation-reduction agent that can produce oxidizing radicals, indirect cross-linking of amino acid residues, and DNA degradation[12], which could interfere with sample analysis.

2. General Anesthesia of the Mouse

1. Choose the anesthetic agent and administration route. Consult a veterinarian from an animal resource facility or Institutional Animal Care and Use Committee (IACUC) regarding potential interference of medications with the immune system and research goals. Some medications may influence cytokine profile expression in animals, including rodents[13]. Use injectable tribromoethanol for terminal procedures or isoflurane for survival endoscopies, along with appropriate controls.
2. For induction, administer 4-5% of isoflurane in 100% oxygen at a rate of 0.2-0.5 L/min; use 0.5-2% for maintenance. This protocol is not appropriate for all animals. Consult your facility veterinarian for proper instructions and training. Abide by relevant IACUC recommendations to achieve the highest standards for animal welfare. Make sure that the mouse if fully anesthetized.
3. 'Strap' the anesthetized mouse to a supporting surface table in a dorsal or ventral position using laboratory adhesive tape. Beware of the anatomical orientation on your endoscope screen. Secure its lower extremities and tail. A band of tape can also be placed over the chest to prevent movement away from the respirator, but avoid restricting thoracic ventilatory movements.

3. Detailed Endoscopic Examination

1. Look for perianal lesions before inserting the endoscope into the rectum, then advance the scope through the anus.
2. Coat the endoscope with sterile surgical lubricant. Be sure not to coat the surface of the CCD (charge coupled device) sensor.
3. Insert the endoscope and start recording. Recorded videos are useful for meticulous evaluation of endoscopy if needed after the examination. Try to perform the endoscopy in the least possible length of time to minimize intestinal/mucosal irritation.
4. Apply air by using a syringe to slowly set apart the intestinal walls. Depending on the size of the mouse, use 1-2 ml of air. Avoid excessive air insufflation; it may cause respiratory distress and death of the anesthetized mouse.
5. Advance the endoscope forward as you clear the way with air or gentle rotation of the scope. Do not advance the endoscope if you do not see the intestinal lumen. Perforations or rectal/colonic tears can occur at this stage, especially in young mice (<8-10 weeks old). Take pictures as necessary.
6. As the scope advances, assess if there is spontaneous bleeding (defined as naturally occurring mucosal hemorrhaging not associated with traumatic endoscopy). Spontaneous bleeding can be observed as the endoscope enters the rectum and colon.
7. Assess the transparency of the colonic wall. Transparency is defined as the ability to visualize the intramural blood vessels in the colon and that of the other surrounding viscera (**Figure 4**). Gentle motion of the scope (back-forward, clockwise, counterclockwise) will help determining if blood vessels can be seen in the context of other viscera. Mesenteric adipose tissue can also be seen. Note if the rectum inside the pelvic cavity appears less transparent than the colonic wall in the abdominal cavity.
8. Inspect to determine the type and number of focal lesions (edematous areas, erosions, ulcers, tumorous masses). This will be recorded in the scoring form using the colonic inflammation score and the decimal identifiers that have been developed.

9. Once the area where the colon flexes caudally into the proximal colon is reached, withdraw the endoscope slowly and look for the presence of contact bleeding (which occurs due to mucosal friability after the endoscope is advanced or removed). It is not necessary to scrape the colon surface intentionally. Healthy colorectal mucosa does not bleed after proper endoscopic examination.

4. Biopsy

1. When biopsies are needed, insert the biopsy forceps through the endoscope channel dedicated for instrumentation. Monitor the advancement of the forceps on the endoscope screen to prevent intestinal perforation.
2. Open the biopsy cups of the forceps to about a 60-80° cup-to-cup angle, and direct them to the area of interest. Excessively opened forceps or pressure may grasp extra layers of intestinal tissue, which results in a biopsy-induced perforation of healthy transparent intestinal walls. If perforation occurs, euthanize the animal during anesthesia following your IACUC recommendations. Perforations result in endotoxemia and peritonitis.
3. Close the biopsy cups, and pull them out while keeping the forceps closed.
4. Remove the specimen from the forceps by flushing the tissue with a solution that is gentle to the tissue and the forceps. RNA later and PBS are common working solutions; if needed, use the needle to dislodge the sample. Avoid eroding the forcep cups or cutting edges.

5. Recovery from Anesthesia

1. Upon completion of the examination, provide a warm environment to the mouse until it recovers. Use either an electric light, or a warm pad set at 37 °C. Avoid overheating; peripheral vasodilation compromises animal recovery. Monitor the mouse closely until full recovery.

6. Integrated Endoscopic Scoring System of Colorectal Health with Decimal Identifiers

1. Use the scoring form (**Figure 2**) and the parameter definitions below (**Endoscopic Assessment of Murine Colorectal Inflammation and Tumors Using a Decimal-weighted Scoring System: Parameter Definitions, and Protocol**) for examples and the optional 'Distal-Proximal Endoscopic Colitis scoring system' (D-PECS) as guidelines for nonbiased assessment and detailed record keeping.
2. Score the four parameters of colorectal inflammation described on the scoring system form (**Figure 2**). Meticulously evaluate the perianal region, intestinal transparency, type of intestinal bleeding (if present), and presence and quantity of focal lesions (erosive, ulcerative, and/or tumorous).
3. When assessing tumors for research purposes, examine the quantity, location, and size, and describe them using the colonic map and bar graph on the scoring form. The numerical sum of the scores obtained and number of lesions (when applicable) will determine the background colonic inflammation, the most predominant type of lesion, and the number of lesions.

Representative Results

The protocol and images presented in this study were prepared with an Olympus URF-V flexible endoscope[14] and NBI[11,15]. Video images were saved in MPEG-2 format and pictures in JPG, TIF, PNG, or DICOM format with maximum 1,280 x 1,024 pixels, transferable to standard USB flash portable memories. NBI enhances the visual contrast of hemoglobin-containing tissues. Normal light and NBI imaging are key features to illustrate the normal anatomy of the colorectal mucosa and the differences between normal and abnormal vascularization during ulceration and tumor development (**Figures 3A** and **6A**). Images with methylene blue as contrast medium during chromoendoscopy show its benefits for close-up images, revealing the aerial normal honeycomb appearance of the colonic crypts (see close up in **Figure 3B**).

To date, we have conducted over 500 murine endoscopies following this protocol, with about 10% representing repeated examination. Generally, the procedure requires 10-15 minutes to complete, split evenly between administration of anesthesia, endoscopy, and recovery from anesthesia. The examination covers from the anus to the splenic flexure of the distal colon. We have also successfully conducted postmortem endoscopic examinations of the proximal colon, cecum and ileum in some cases, following postmortem dissection of the tissues immediately prior to harvesting tissues for histology (**Figure 6B**). In one case, we also observed and confirmed suspected gastric dilation in a mouse affected with severe duodenal stricture and impaction due to intestinal adhesions. The abnormal image was observed transmurally from the most cranial aspect of a mildly inflamed distal colon; postmortem examination showed the distended stomach was juxtaposed to the colon.

We have developed a valid and reliable decimal-weighted scoring system to improve the standardized application of murine endoscopy across published research studies. Three scoring systems have been previously employed and served as the foundation for the development of our decimal-weighted system[4-6]. Although these systems allow for the categorization of various criteria and levels of lesion severity, they lack proper criteria definitions, some of which are difficult to evaluate from endoscopic images. In some cases, ambiguous parameters included in the scoring systems have questionable relevance to endoscopic examination in mice. A summary of eleven published parameters[4-6] and their endoscopic advantages, limitations, and physiological relevance are presented in **Table 1**.

Because the proposed murine colonic endoscopy scoring system will be used for research purposes, its usefulness for performing statistical inference was essential. To assure that the scoring system had high construct validity, we first developed a conceptual framework to identify relevant parameters, grouped these parameters to fewer criteria (n=3), and assessed their colinearity and correlation with actual presence of inflammation and healing (**Figure 7**). Next, we added perianal findings using discrete integers to represent ordinal levels of severity, and integrated the concept of tumor induction and development using decimal identifiers. In addition, we added ‘strictures’ as another parameter not previously considered/reported in murine intestinal endoscopy. The resulting output scores produce high validity and are suitable for parametric statistical analysis. Lastly, we added mapping and numerical plotting capabilities for detailed disease monitoring.

To assess the convergent validity of our assessment system, the scores obtained from three published parameters for 36 mouse colonoscopies were compared against the scores obtained by the same person blindly using three previous scoring systems[4-6]. Every comparison showed good positive correlation, indicating high convergent validity for our integrated scoring system (Pearson $p<0.0001$; **Figure 8**). The analysis supports

the inclusion of perianal findings as part of the clinical criteria to assess colorectal health, as it was commonly present in cases of severe colitis and improved data dispersion (P=0.0351 between score 0 and 1, P=0.0086 between score 2 and 3, **Figure 8B**). When the integrated scoring system (after adding perianal findings) was compared to the other available systems, it was evident that 2 of the 3 other systems have a major inferential limitation due to clustering of cases to one of the four possible integer categories, a major indication of method misclassification bias (**Figure 8C**)[16]. With decimal identifiers, our novel scoring system has the lowest probability of clustering data points in integer categories.

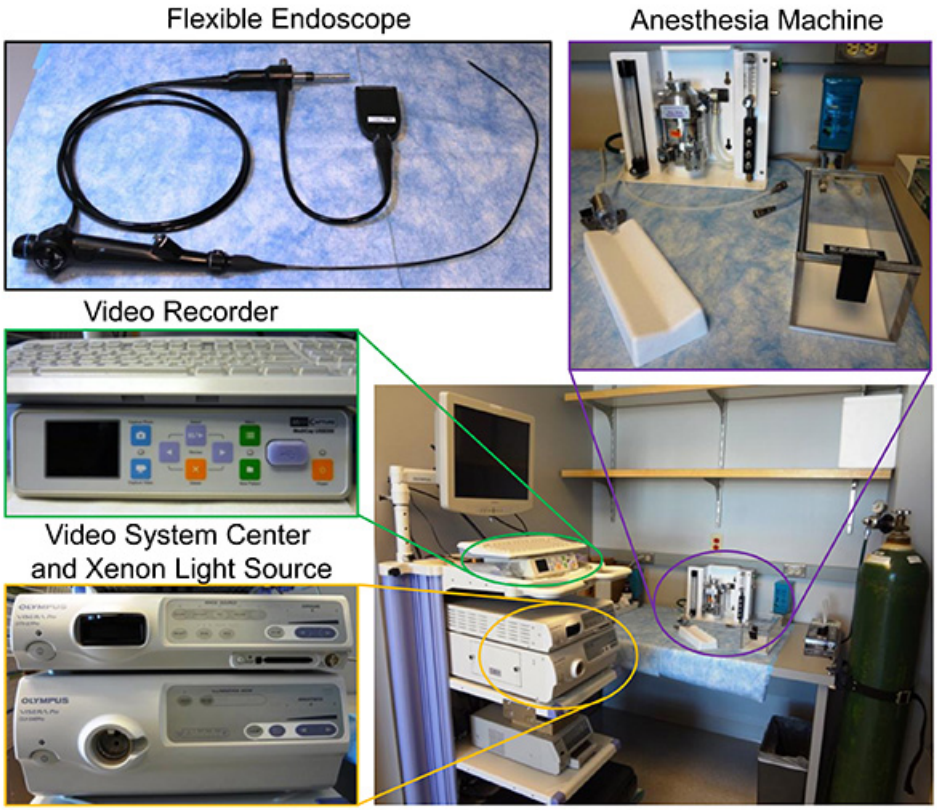

Figure 1. Instruments. Flexible endoscope, video control system, light source, video recorder, and inhalation anesthesia machine used in this study. Click here to view larger image.

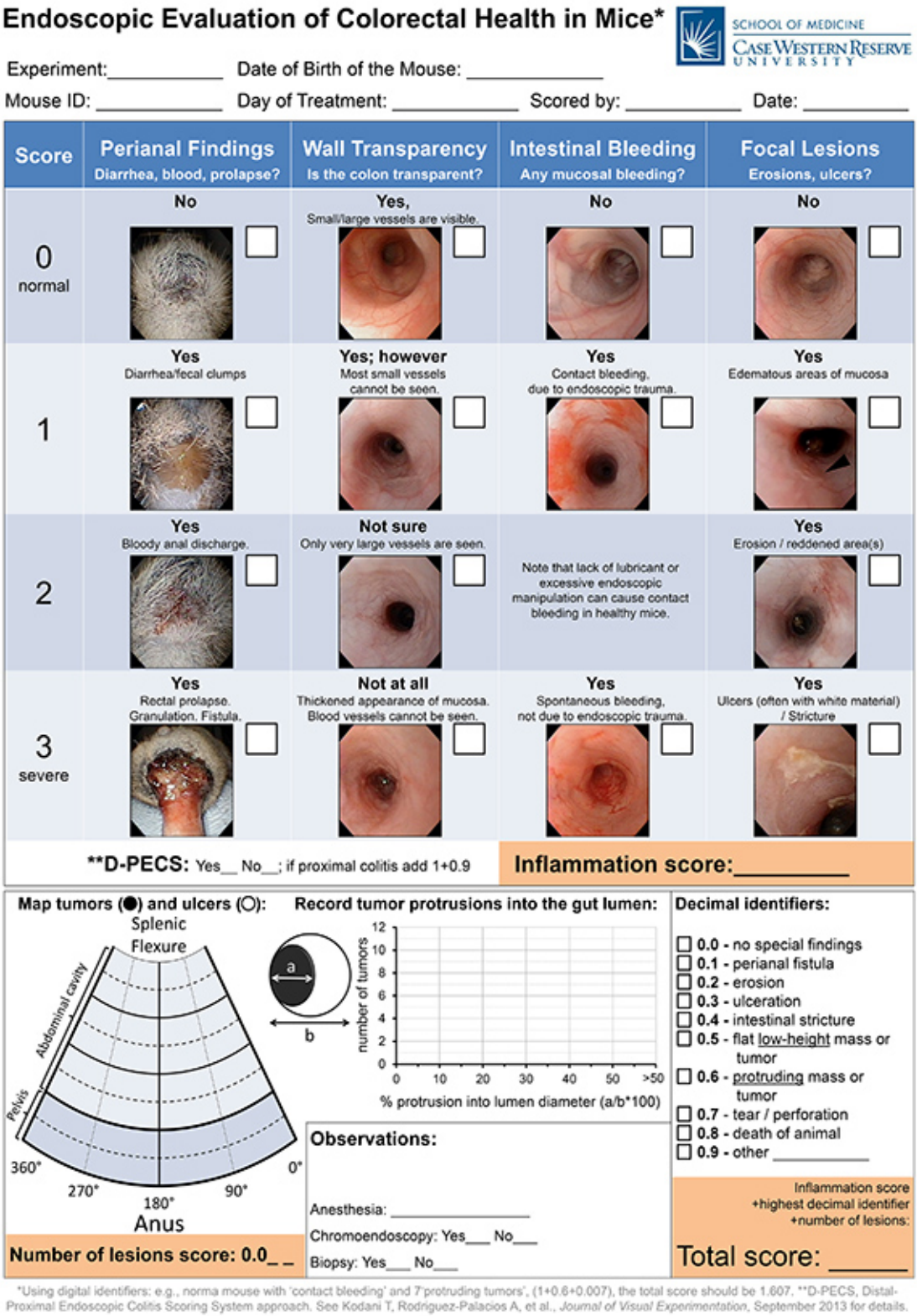

Figure 2. Decimal scoring system form for assessment of colitis and colorectal tumors in mice. The system has a core inflammatory component of four non-co-linear parameters, graphics for tumor information, and decimal units to notable lesions or complications. See detailed criteria and examples in section below (Endoscopic Assessment of Murine Colorectal Inflammation and Tumors Using a Decimal-weighted Scoring System). Click here to view larger image.

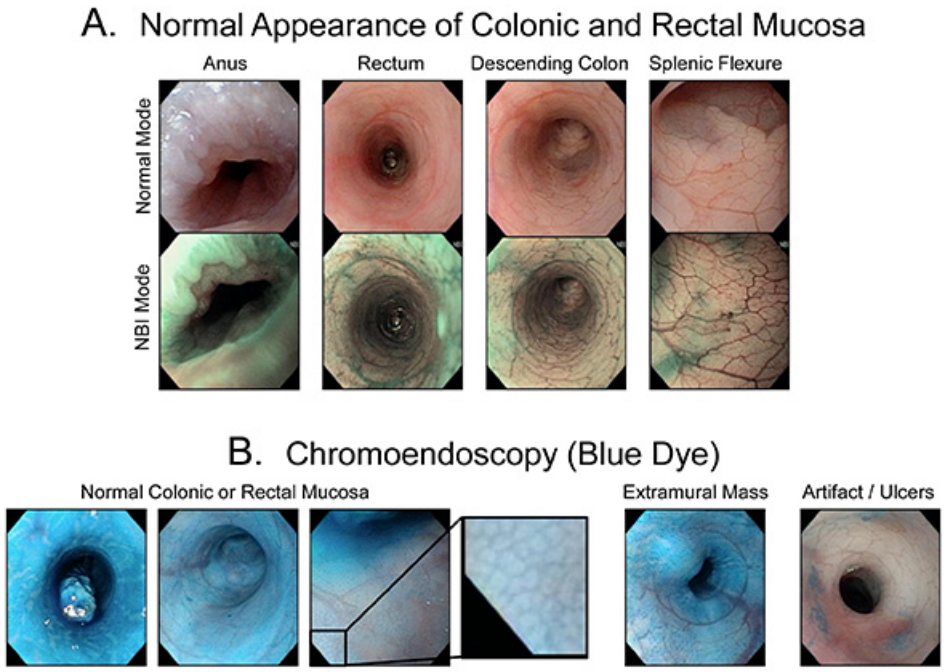

Figure 3. Paired images of normal mucosa in normal endoscopic light mode, NBI mode, and with chromoendoscopy. Arborization of blood vessels is normally less common in the rectum. Click here to view larger image.

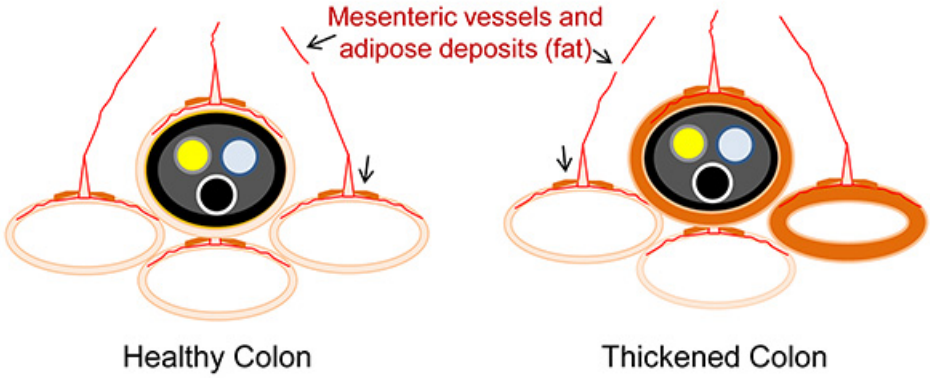

Figure 4. Cross-sectional representation of intestinal loops to illustrate the principle of endoscopic transparency of the intestinal wall in mice. Endoscopic visualization of mural intestinal vessels and mesenteric fat, and that of mesenteric vessels and fat from adjacent healthy viscera, is possible through the wall of a healthy colon. Inflammation and thickening of the intestinal wall in the peritoneal sections of the distal colon prevents the visualization of intramural and transmural structures from other viscera, even if other intestinal segments are healthy. Click here to view larger image.

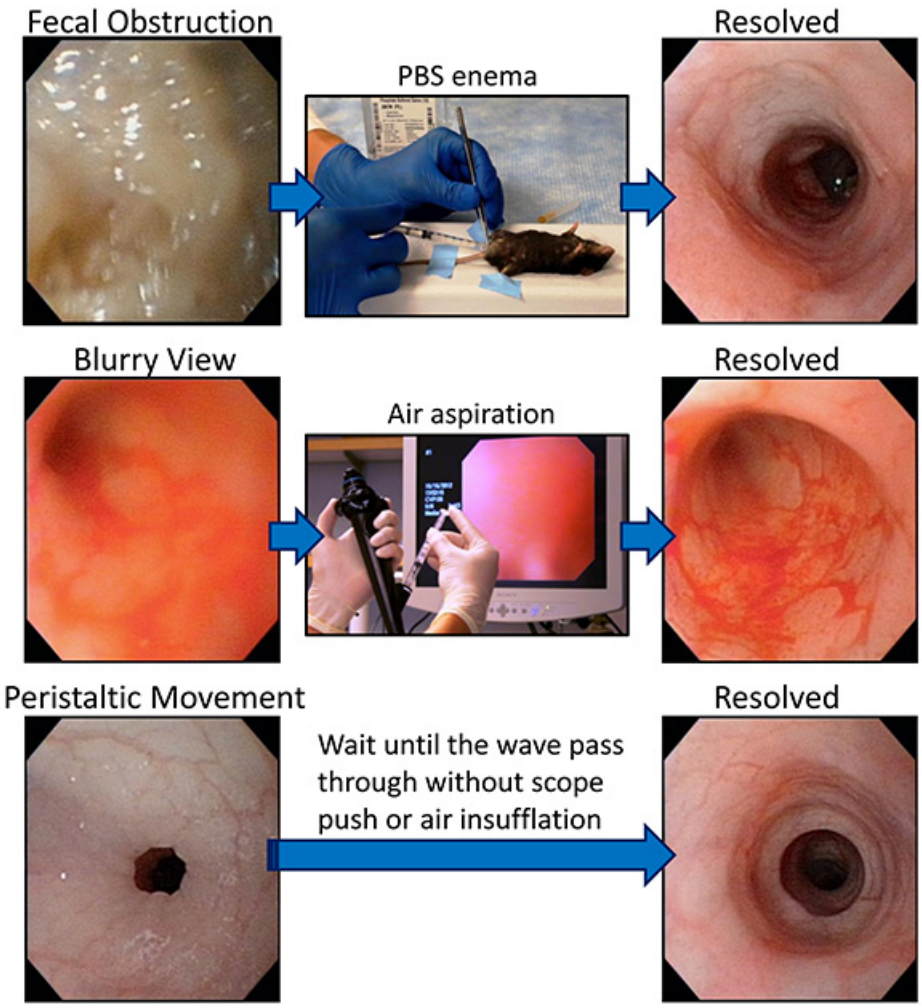

Figure 5. Troubleshooting. Resolving low visibility caused by intestinal content, blurry images and peristaltic movements. Click here to view larger image.

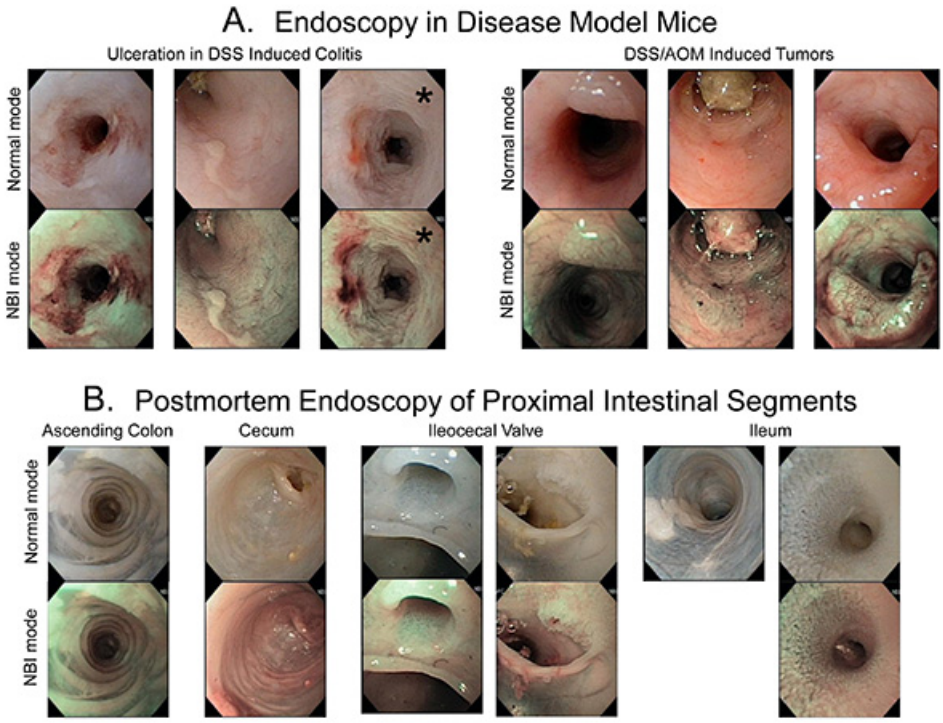

Figure 6. Paired images (regular light vs. narrow-band imaging, NBI) of diseased colorectal mucosa and other proximal intestinal segments. NBI helps to identify ulcerative and tumorous lesions. Some ulcers appear brownish with NBI; others, covered with fibrinous material appear thick and white. AOM/DSS tumors are better characterized with NBI, especially during early stages when they are not protruding into the lumen. Asterisks (*) highlight endoscopic artifact (mucosal corrugation) in colon filled with PBS, not observed in all other images where colon is distended with air. Click here to view larger image.

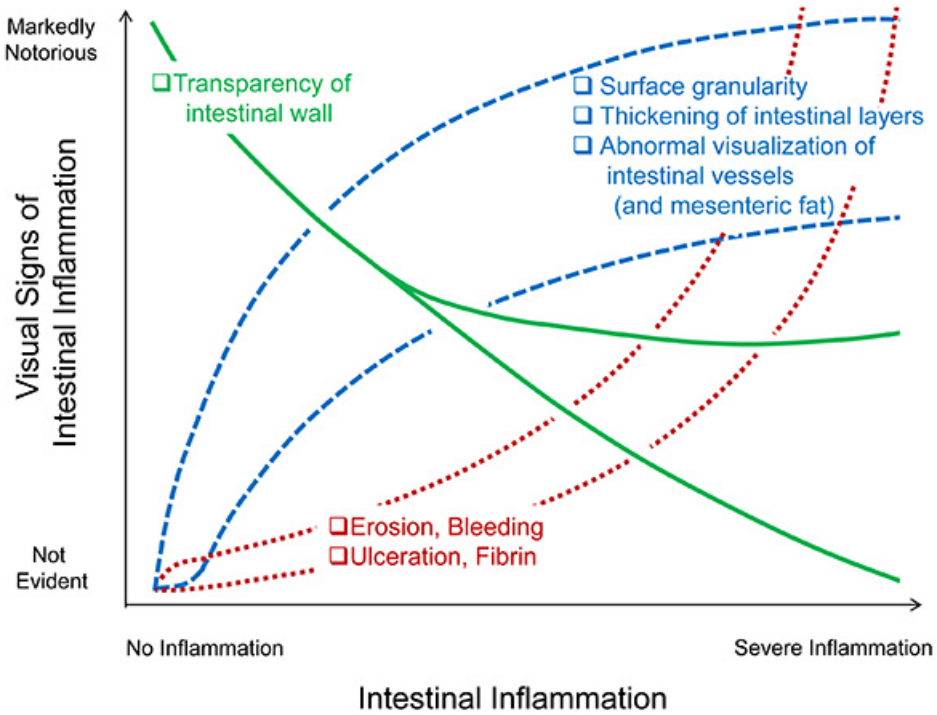

Figure 7. Theoretical correlation and co-linearity between the severity of intestinal inflammation and the ability to visually discern and grade various published criteria of colonic inflammation (see Table 1). Click here to view larger image.

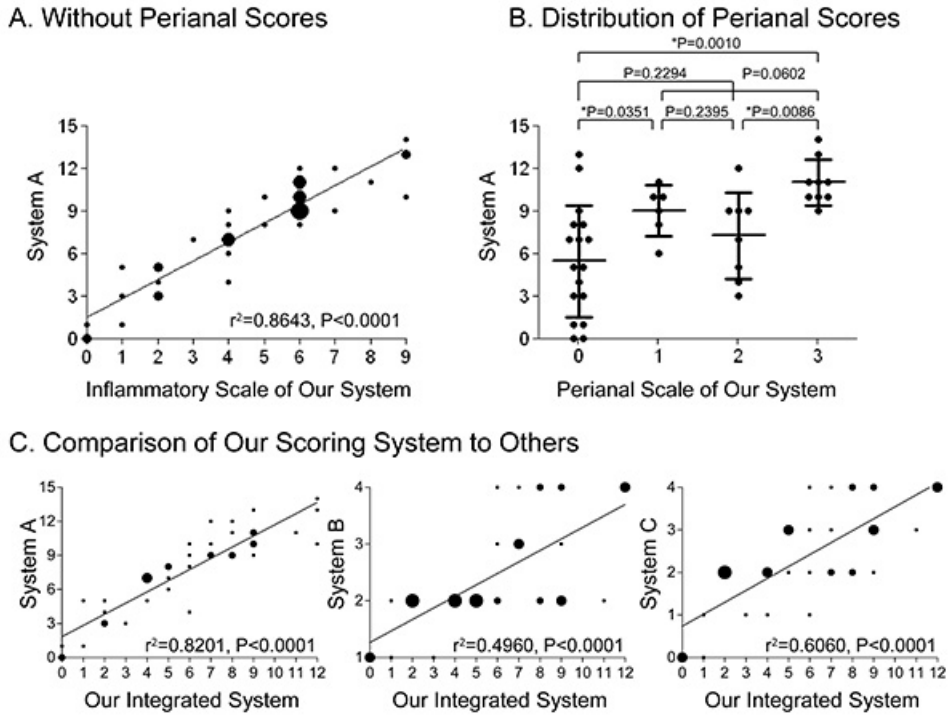

Figure 8. Comparison of performance between our scoring system and previously published scoring systems. Data from paired blind analyses of 36 mouse video endoscopies. **A**) Comparison of System A (5 parameters) to the inflammatory parameters, except the perianal lesions (3 parameters). **B**) Importance of including perianal findings in the scoring system; perianal examination consistently provided additional statistically significant information in cases of moderate/severe colitis in mice. **C**) Performance of the scoring system after including the perianal findings in comparison to previously reported systems (36 mouse video endoscopies were scored with each method). Note that systems B and C have a major limitation due to clustering of cases in one of their four categories leading to misclassification bias (Systems B and C cluster cases in nonequivalent categories). By adding decimal identifiers, we also lowered the chance of clustering in integer categories, while allowing tracking of now meaningful numerical scores. Click here to view larger image.

	Wall Thickness and Blood Vessels					Mucosal Surface			Mucosal Bleeding		Feces	
Reference	Thickening	Edema	Loss of Vascularity	Abnormal Vascular Pattern	Granularity	Fibrin	Erosion	Ulceration	Contact Bleeding	Spontaneous Bleeding	Stool Consistency	Criteria, n=, /maximum possible score
Becker, 2005	X		X	X	X	X				X	X	7/15
Ravnic, 2007		X			X			X	X	X		5/4
Hamilton, 2011			X				X	X	X			4/4
Our comment, from an endoscopic stand point	Distinguishable endoscopically, but requires proper definition criteria			Highly subjective if not accurately defined		Difficult to define	Sometimes difficult to distinguish erosion from ulceration.		Clearly distinguishable, extent of contact bleeding depends on endoscopic manipulation		Inappropriate for endoscopic evaluation	Potential for selection bias
Functional relevance to colon health	Vascular permeability, inflammatory cell infiltration, angiogenesis					Bleeding/ healing	Mucosa damage/ infection		Mucosa fragility		Colonic water reabsorption	Inferential statistics

Table 1. Clinical parameters published as endoscopic criteria to assess intestinal inflammation in mice. Click here to view larger image.

Endoscopic Assessment of Murine Colorectal Inflammation and Tumors Using a Decimal-weighted Scoring System: Parameter Definitions and Protocol

Our integrated endoscopic scoring system for colorectal health has three major components: 1) assessment of the extent and severity of colorectal inflammation, with four parameters and 12 possible numerical grades; 2) recording of the location, size and number of tumorous lesions; and 3) decimal units with assigned types of primary or predominant lesions and endoscopic complications to highlight findings of special significance for the researcher, herein referred to as “decimal identifiers”. The location and size of tumors are recorded on a virtual colonic map, and the number of tumors in each percentage of protrusion (obstruction) into the lumen is recorded on a graph. These two charts provide tumor information that can be integrated in three-dimensions. The decimal units for lesion categories range from 0.0-0.9. If the number of lesions for a particular category is relevant, second and third decimal places can be used to indicate the number of lesions. For example, if three lesions are observed, adding 0.003 would allow the researcher to know that there were three lesions; if there are 12 lesions, then 0.012 would be used. This tracking system is of special benefit if repeated endoscopic examinations are anticipated. Detailed definitions of the inflammation criteria and severity grades are given below.

A. Assessing colorectal infammation with four clinical parameters - definition of endoscopic criteria

1. Perianal findings: Assess this aspect before commencing scoping to avoid confusion with post-endoscopy bleeding. Health of the perianal region is a proxy for health status of the colon, skin, and the animal's attitude in general, and is included as part of the endoscopic examination because some lesions cannot and should not be examined closely with the naked eye. Exercise biosafety measures as appropriate. Note that healthy grooming habits by the mice may remove blood/discharges, but will not remove skin lesions from the perianal region. Scoring:

0. Normal. No blood, diarrhea, fissures, fistulas or discharge observed.
1. Clumps of fecal matter or diarrhea observed.
2. Clots/clumps of bloody discharge, loose feces (diarrhea), pus, or minor ulcerations observed.
3. Granulation tissue or lesions observed that involve most of the surrounding skin and indicate that the animal has marked irritation or inflammation in the perianal region; rectal prolapse.

2. Transparency of the colon: Defined as the ability to visualize the blood vessels or extramural organs through the colonic wall as the colon-endoscope is gently moved. Note that the rectum is naturally less transparent due to the muscular layers and because it is in the pelvic cavity where less contrast exists. This is one of the physiologically most comprehensive parameters as it reflects the overall inflammatory condition or health of the murine colon. Note that it is possible to have a non-inflamed colon with mucosal erosions/ulcers in early stages of infection or toxic effects on the epithelium. Beware that physiological isotonic solution should be used for flushing during endoscopy, as hypotonic solutions, such as water, cause changes with whitening and contraction of the mucosa in mice if use for prolonged periods (See asterisks in **Figure 6**).

0. Normal. Large and small blood vessels of the colon and the surrounding viscera are clearly visible. Move the endoscope slightly (back and forth, clock/counterclockwise). White mesenteric fat or dark content of other viscera can also be observed.
1. Small vessels are partially invisible but large vessels are visible.
2. Small vessels are invisible and large vessels are not clear. The mucosa does not appear thickened.
3. Apparent thickening of the intestinal wall and/or mucosal edema/generalized. Blood vessels are not visible. Few distended/varicose vessels may be visible in some cases.

3. Mucosal bleeding: Differentiate contact bleeding from spontaneous bleeding. Routine standard endoscopic procedures in healthy mice should not induce bleeding. If there is inflammation, the increased mucosal vascularity and tissue fragility can lead to mucosal erosion and bleeding. There is no need to intentionally scrape the colon to assess this parameter.

0. Normal, no bleeding.
1. Common contact bleeding. This traumatic bleeding is defined as contact bleeding; it should be judged only after withdrawing the endoscope.
2. Not applicable. Intentionally not assigned to force the examiner to decide the nature of bleeding.
3. Spontaneous bleeding, defined as a non-traumatic and naturally-occurring hemorrhage that is observed as the endoscope enters the colon. Sometimes, air insufflation induces spontaneous bleeding during severe colitis.

4. Focal lesions: To accommodate the patchy, unpredictable nature of colorectal diseases, this integrated scoring system allows grading for the presence of focal lesions (mostly ulcerative in nature) by the severity of the inflammation. Since the colon is a short, straight tubular segment in mice, it was deemed unnecessary to describe the location of the lesions. Instead, the number of lesions are recorded and added to the total score using the system of decimal identifiers. However, if emphasis on the location of the lesions is needed, we have an option called 'Distal-Proximal Endoscopic Colitis Scoring System' (D-PECS) described below in section F. Further streamlined applications can be derived from our decimal scoring system. Because tumors rarely exist without inducing some form of inflammation in the colorectal region in mice, we have included tumors in the decimal identifiers section to uniquely allow the study of tumors in the context of intestinal health (see below).

0. Normal, no focal lesions.
1. Well-defined areas of small flat elevation covered by normal mucosa observed. This is to address local edematous areas or potential areas of cell infiltration/proliferation, as found in mild colitis.
2. Reddened or erosive areas observed.
3. Ulceration or strictures. Ulcers are identified by redness and deformity (irregular edges with evidence of abnormal vascularization) due to edematous or fibrotic wound repair. NBI endoscopy is useful to differentiate detection or observation of ulcerations. Strictures are recognized as areas abnormally narrow, circular or irregular and static in shape, that do not contract or expand as expected. Often strictures involve areas of abnormal mucosal crypts, or villi in the ileum. In the case of flexible endoscopes (external diameter = 3.3 mm) the careful inability to advance the scope into more proximal areas of the colon has been consistently associated with abnormal strictures verified post-mortem.

B. Tumor charts for recording location, size and number

1. Virtual colonic map: This map is a virtual perspective image of the distal colon and rectum that is made by cutting the top of the lumen (0°) and rolling it out. The top and bottom of the chart are assigned proximal of the colon (flexure), and anus, respectively. The center line (180°) indicates the bottom of the lumen (ventral aspect of animal), and right and left halves of the semicircles indicate the right and left wall of the lumen. The map can be used to describe the location and horizontal size of tumors, as well as ulcerations.

2. Tumor numbers for each degree of tumor occupation: Although the colonic map provides only two-dimensional information, the extent to which tumors grow into the gut lumen is an important feature of tumors, since protrusion can cause intestinal stenosis or obstruction. To capture this information, we added a recording graph for percent protrusion into the lumen that documents the number of tumors in each percentage range, allowing a three-dimensional perspective for assessing the extent of colonic tumors.

C. Decimal identifiers to make numerical scores meaningful and fast-track relevant cases

Our scoring system uses a novel decimal identifier system to record the presence of relevant lesions or complications. These identifiers are for descriptive purposes and are intended to facilitate interpretation of a numerical score and fast-track cases of endoscopic interest. However, they also serve to numerically weight predominant types of lesions, and allow the generation of data sets amenable to parametric statistics. When using the developed scoring form, mark the lesions as appropriate, and choose one decimal identifier to be added to the inflammatory score.

0. No special findings.
1. Perianal fistula, or excessive perianal inflammation, rectal prolapse.
2. Primarily erosive lesions with minor wall involvement.
3. Mostly ulcerative lesions, some covered with fibrin, bleeding, or large with irregular shapes due to coalescing of small ulcers (the number of ulcers can be recorded as 0.301 for one tumor, 0.302 for two tumors, 0.311 for eleven tumors, *etc*.).
4. Intestinal stricture (optional because stricture is a severe inflammatory complication in colitis, add 2 points to the total inflammatory score).
5. Well-defined plaque (thick/nontransparent tissue within intestinal wall; *e.g.* a flat low-height tumor). The number of plaques can be recorded as described for lesions (*i.e.,* 0.401, 0.402,...0.411, *etc*.). They may appear as a flat non expandable surface that may seem to protrude up to about 5-10% which modifies the circumferential appearance of the intestinal lumen.
6. Protruding mass or tumorous lesion. The number of masses can be recorded as described for lesions (*i.e.,* 0.601, 0.602,...0.611, *etc*.). They are clearly bulging, but may appear flat but protruding >15% of the distended circumference of the intestinal lumen.
7. Induced tear/perforation during endoscopy.
8. Death of animal during endoscopy.
9. Other: _____ (*e.g.* severe proximal endoscopic colitis, see Distal-Proximal Endoscopic Colitis Scoring System below).

D. Examples and applications

The murine endoscopy scoring system presented in this paper is specifically designed to integrate the collection and analysis of data on colonic inflammation and colorectal tumors. With respect to tumors, the data can be analyzed using multiple statistical approaches, including area under the curve, median, mode, or principal component analysis. At the individual level, the scoring system can help researchers monitor colonic health in a single mouse over time. After summing the inflammatory subscores and appending the proper decimal identifier for type and number of lesions where appropriate, the total score will allow researchers to numerically quantify the extent of colonic inflammation, the most predominant type of lesion, and the number of lesions. For example, a score of 12.609 indicates that this mouse has severe colorectal inflammation and nine protruding tumorous masses; a healthy mouse with a normal perianal region and colorectal mucosa that was perforated during endoscopy would have a total score of 0.7. The decimal identifier system will allow researchers to catalogue and easily search large data repositories of scored mice. For example, a researcher could set search criteria to identify all scores that end in “0.6” and retrieve all cases that have protruding tumors. More examples are illustrated in the supplementary Figures S1-S3.

E. Distal-proximal endoscopic colitis scoring system (D-PECS)

This is an optional modification of our decimal identifier scoring system to differentiate cases with severe/extensive proximal colitis. Because the endoscopic signs of colitis and colorectal tumors in mice appear to develop consistently in the distal segments of the colon we recommend:

- To score the colon following the criteria described, paying special attention to the distal two-thirds of the colon and ignore the proximal segment if it is apparently healthy. This avoids confusion and increases discriminatory power of endoscopy to differentiate animals with various degrees of extensive colitis.
- If inflammatory or tumor changes are observed in the proximal third, we recommend to add one point to the total score (1.0) and assign the decimal identifier as ‘0.9 - Other,’ which could be labeled as severe proximal endoscopic colitis. Plot the findings in the map grid and bar chart.
- Monitor animals that have endoscopic abnormalities in the proximal third of the colon. Pay special attention postmortem, as disease may be unusually severe or may extend beyond the splenic flexure towards the proximal colon.
- If this recommendation was followed, record this approach in the ‘observations section’ of the evaluation form, and follow this trend across experiments. Please cite in your final publication that the assessment was conducted following our ‘distal-proximal endoscopic colitis scoring system’ or D-PECS, which ranges from 0-13.9. Note that this modification has no statistical effect on our scoring system if used in animals without extensive/severe proximal colitis.

Supplemental Figures.

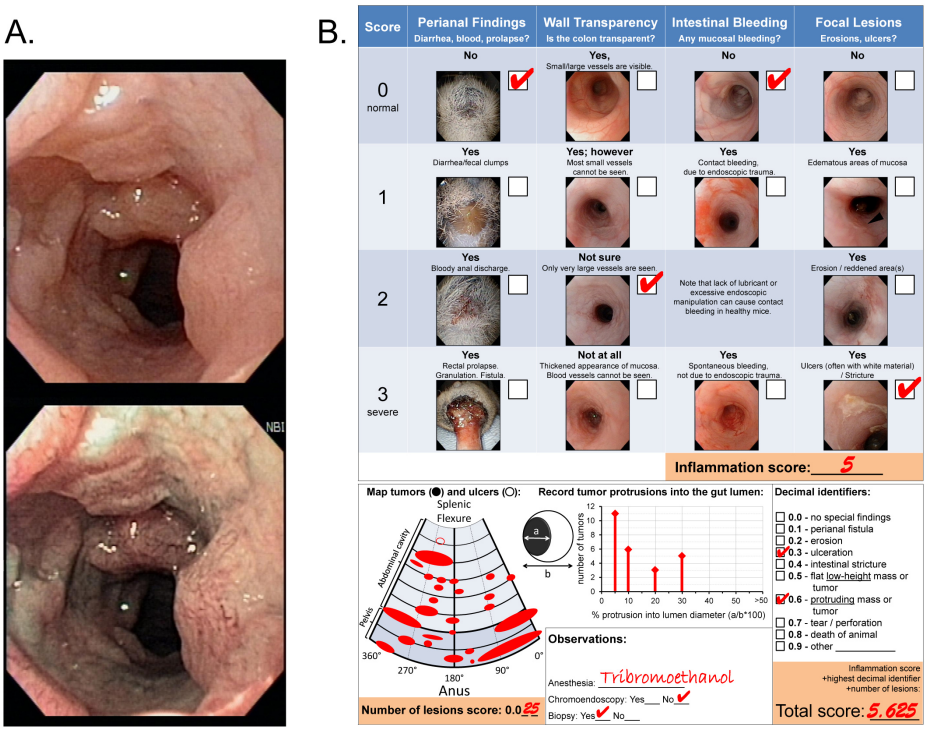

Figure s1. Example of endoscopic assessment of a mouse with colorectal tumors. Animals were treated with AOM and DSS prior endoscopy. **A)** Representative image in normal and NBI mode, respectively. **B)** Scoring form filled with endoscopic findings. Note that although there are two relevant findings, the researcher chose to assign the decimal identifier 0.6 based on clinical severity and research goals. A variety of parametric statistical analysis can be conducted with the data recorded in the 'Map of Tumors/ulcers' and the 'Record of tumor protrusions' charts. Click here to view larger image.

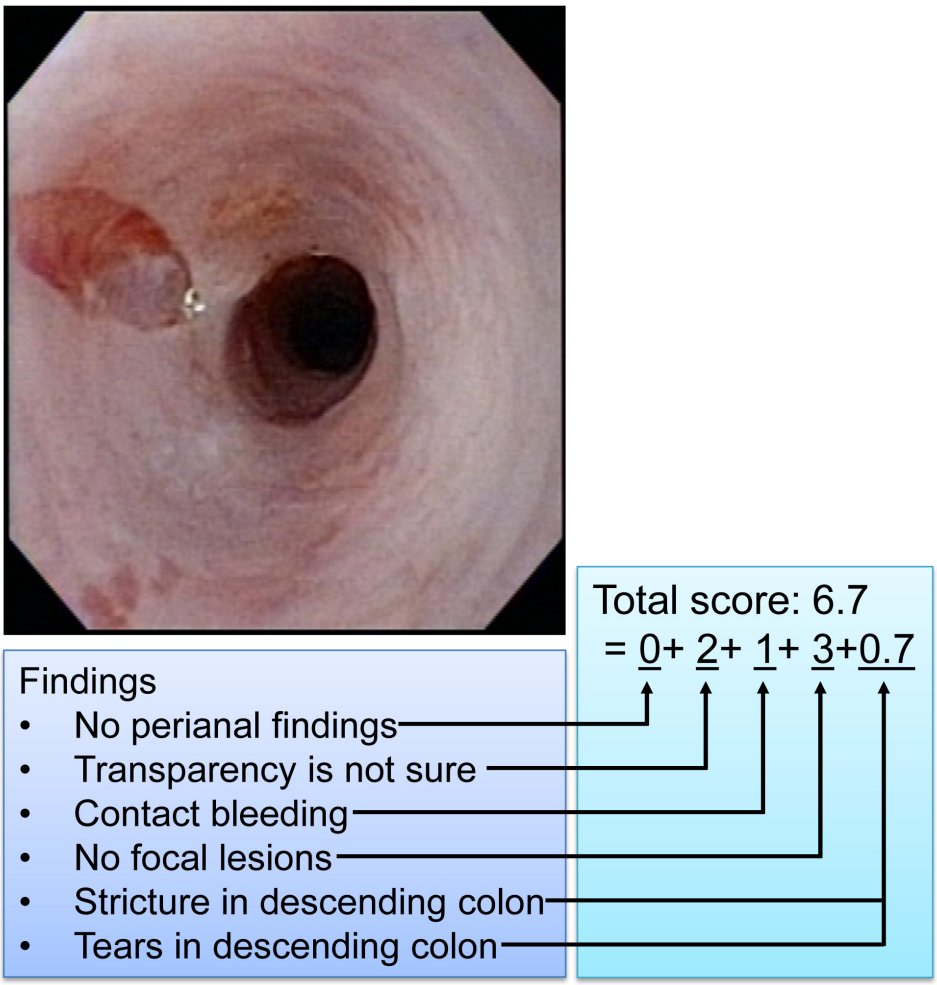

Figure s2. Example of a mouse with a colonic stricture and an endoscopic complication. Note the narrowing of the intestinal lumen and that a nonperforating, mucosal tear was induced during endoscopy just distal to the stricture site. Narrowing and debilitation of the inflamed colon may result in mucosal tears. The animal was under general anesthesia. Note that the scoring system has allowed us to monitor and reduce procedural complications. Click here to view larger image.

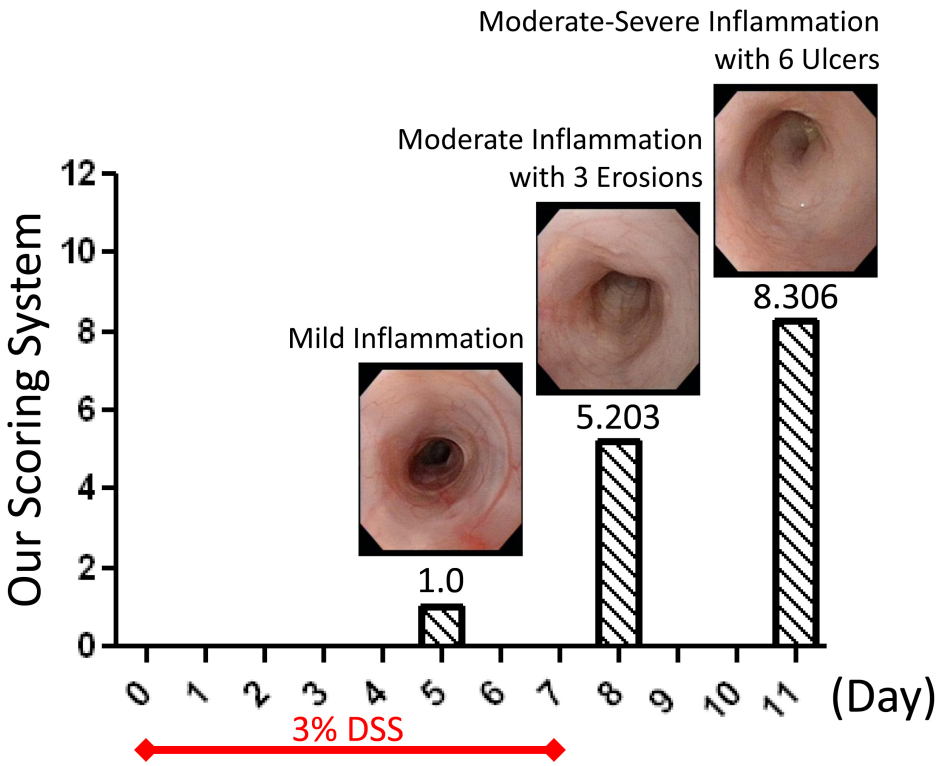

Figure s3. Example of time course of inflammation and endoscopic scoring in a DSS-treated mouse. Note that the numbers can be self-explanatory and indicate that colonic inflammation progressed (from 1-5 and 8), and that erosions developed by day 8 (decimal 0.2) and ulcers by day 11 (decimal 0.3). Click here to view larger image.

Discussion

There are several considerations regarding modification and troubleshooting of the endoscopic technique in mice. Critical aspects of the procedure that need to be mastered during the use of flexible endoscopy include the adjustment of the air volume needed to prevent abdominal distension and respiratory compromise of the mouse, and minor technical aspects of view control with coordination of the angulation and torque controls. Torque control is important to minimize the risk of intestinal perforation. When handled properly, murine flexible endoscopy becomes a very safe, rapid procedure that allows direct visualization and biopsy sampling of intestinal areas of research interest.

To solve view obstructions due to fecal matter (**Figure 5**), it is important to understand that fecal pellets are usually observed even after fasting or the use of laxatives. When present, feces can be easily moved proximally with gentle air insufflation. In about 11% of cases, it is necessary to also use 1 ml of PBS enema. Prior to conducting the enema, exercise caution when examining animals that seem to have abdominal distention or that are pregnant. For peristaltic movements it is important to wait a few seconds until the peristaltic wave passes since air insufflation does not stop peristalsis.

To date, most endoscopic assessment systems used to evaluate murine intestinal disease are specific for either colitis or cancer. Given that these two conditions often occur concomitantly and can interact with one another, a standardized scoring system that takes into account both inflammation and tumor formation would greatly facilitate cross-discipline communication, thereby enhancing the usability of information gained during gastrointestinal research by cancer researchers, and vice versa. Because tumors rarely exist without inducing some form of inflammatory alteration in the colorectal region in mice, we have included tumors in the decimal identifiers section of our scoring system to uniquely allow the study of tumors in the context of intestinal health. To enhance the validity and reliability of the scoring system and facilitate its use in long-term experiments or clinical trials, we also developed a standardized report form to use together with the scoring system (**Figure 2**), which systematically stores the data elements required to calculate murine intestinal inflammation scores, based on precise definitions for each classification criteria.

The previous availability of multiple murine endoscopy scoring systems of limited validity hinders interpretation of results across studies. To minimize the confusion experienced by laboratory personnel in deciding which published method to use, we recommend performing endoscopic assessment using criteria that can be inferred from two-dimensional images (*e.g.* avoid criteria that refer to texture or consistency - of feces; note that some mice including germ-free may have soft stools), and that are not co-linear (*i.e.* that are physiologically and temporally driven by the same inflammatory context) Because the cardinal signs of inflammation are sequential due to chronological inflammatory events, we recommend the following criteria: 1) intestinal transparency, first described by Becker *et al.*[4], as a reflection of wall thickness; 2) bleeding, as an indicator of mucosal and blood vessel integrity; 3) perianal findings, as an indicator of colonic integrity and skin irritation or predisposition to excessive inflammation, and 4) focal lesions, to grade the severity of inflammatory lesions (which are in mice primarily ulcerative). To date, we have not seen tumorous lesions in the typical models of 3% DSS-induced murine colitis. The murine endoscopic scoring system described in this

paper provides for the first time, to our knowledge a quantitative mechanism for evaluating the presence and induction of tumorous lesions in the context of colonic inflammation.

For the first time, we also highlight the importance to include strictures as a parameter during murine endoscopy, since they appear to be commonly present in certain animal models of intestinal inflammation, *e.g.* SAMP/Yit/Fc. Strictures represent (semi)circumferential areas of submucosal chronic inflammation, with loss of intestinal elasticity due to increased fibrosis, which are difficult to assess endoscopically for the novice. We have noticed in endoscopically defined stricture-affected mice, that there is more inflammation in segments that are proximal to the stricture as compared to the distal segments (which can be examined endoscopically), likely due to slowed intestinal transit. For research purposes, it is advisable to follow up animals histologically at the end of experiments to better characterize the inflammatory responses that flank candidate stricture regions.

Without proper training and expert feedback, any scoring system may have variability at any given time. It is important to emphasize that endoscopic training procedures conducted on mice should be monitored by a trained veterinary of medical professional to ensure: i) proper training of other scientists or technicians, and ii) to ensure that animals are not misdiagnosed or inadvertently injured during the procedure. Consult the veterinarian in charge of your animal research facility for expert advice before proceeding to implement this procedure to ensure the best possible practices and animal welfare. It is advisable to routinely monitor the performance of trained endoscopists. Use the images we have presented herein as guidelines, randomize the order in which animals are evaluated, and assess every animal in a blinded fashion. Consult the parameter definitions and protocol criteria periodically and keep them at hand during endoscopy and scoring **Section 6)**.

In summary, as a team of medical and veterinary gastroenterologists, we present a standardized protocol and semi-quantitative scoring system for endoscopic evaluation of murine models of colitis and tumors. These research tools will enable researchers to conduct routine murine endoscopies, and will allow research personnel at all levels of experience to generate reproducible and comparable measures of intestinal inflammation and tumor formation across laboratories and between disciplines. We have developed the system to produce meaningful numerical scores with high validity, and provided capabilities for fast tracking of particular findings, which can be customizable for internal use within individual laboratories. We also describe a discriminatory scoring alternative that takes into consideration differences in regional susceptibility to disease within the colon (see Section E, 'Distal-proximal endoscopic colitis scoring system, acronym D-PECS). The data report form and some figures can be used as wall posters to aid inexperienced scientists (**Figures 1, 3, 5, 6**). Successful implementation of murine endoscopy hinges on becoming familiar with the normal anatomy of the mucocutaneous junction in the anus, rectum, colon, and surrounding viscera, as anatomical differences account for subtle endoscopic differences[17].

Although at present even the smallest, most flexible, and finest endoscopic technologies cannot reach the proximal colon *in vivo*, fast moving technological advances in the field of capsule and single fiber endoscopy may become available for mice in the near future. The standardized protocol and the endoscopic scoring system presented in this paper represent valuable tools for visualizing and assessing the status of the murine intestinal environment in live animals. These tools will facilitate comparability of results between papers and across disciplines, and will have applicability to new and emerging murine endoscopic technologies.

Disclosures

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