Dynamic Tissue Perfusion Measurement in the Intestinal Wall — Correlation With Ulcerative Colitis

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Chronic inflammatory bowel diseases are characterized by unpredictable exacerbations, which require an adaptation of medical treatment. The optimal dosage of anti-inflammatory drugs is often determined based on patient complaints. Children are often especially difficult to evaluate, since their complaints tend to be vague and require an independent evaluation by laboratory and endoscopic findings. Colonoscopy, however, cannot be repeated too often since it is invasive, requires sedation and raises the threshold for a precise diagnosis of actual disease activity. Dynamic tissue perfusion measurement is a non-invasive method of blood flow intensity evaluation, which has been successfully used to grade inflammatory activities in children with Crohn's disease. We investigated the correlation of histological findings in ulcerative colitis and local bowel wall perfusion in affected parts of the colon at the site of biopsy. We found a significant correlation between intestinal wall perfusion and the score of lymphocyte infiltration, crypt abscesses, neutrophil infiltration, as well as an inverse correlation with wall edema. Subjects included 12 pediatric patients with ulcerative colitis from whom biopsies were taken and analyzed. Dynamic tissue perfusion measurement is a useful and patient-friendly adjunct to the conventional diagnostic armamentarium, which reduces the use of colonoscopies.

KEY WORDS — bowel wall, chronic inflammatory bowel disease, dynamic tissue perfusion measurement, ulcerative colitis

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Introduction

The main method for diagnosis of chronic inflammatory bowel disease (IBD) is the histological examination of intestinal wall biopsies from affected bowel segments. The unpredictable course of these inflammatory diseases requires frequent adaptations of medication to the actual activity of the inflammation. The medication needs to be aggressive enough to suppress inflammation while pharmaceutical side

effects must also be considered. The invasiveness of colonoscopies sets a high threshold for their use, especially in children. Therefore, pharmaceutical treatment relies on indirect parameters of inflammatory activity.

A variety of activity indices has been introduced to assess the actual disease activity in IBD, Crohn's disease (CD) [1] and ulcerative colitis (UC) [2,3]. The sheer number of these activity indices reflects their inherent flaws and the lack of agreement among researchers as to the appropriateness of the individual components of these indices. For CD, the dynamic tissue perfusion measurement (DTPM) is correlated with the inflammatory activity in general. Moreover, DTPM refers to data measured directly at the site of inflammation and reflects the course of inflammation over time more precisely than the pediatric CD activity index [4]. Most activity indices indicate relapses [5] or aid in the decision for colectomy [6]. UC activity indices are based partially on bioptic results, thus making a purely non-invasive approach to disease activity impossible [7]. Attempts to predict UC activity from saliva concentrations of transforming growth factor-beta or nitric oxide have been unsuccessful [8].

We hypothesized that a local measurement of inflammatory hyperperfusion by means of DTPM accurately reflects local inflammation in UC. If this hypothesis is correct, a direct calculation of disease activity could be recommended, thus ruling out sets of indirect parameters.

Materials and Methods

Patients
In 12 children and adolescents (aged 12 to 17 years), 17 colonoscopies were carried out. A total of 114 colon biopsies were taken with an interval of less than 4 weeks after a standardized sonographic examination of the intestine prior to initiation of treatment. These biopsies were classified according to a scoring system encompassing relevant histological criteria of acute and chronic inflammation (Table 1 [9–23]).

Duplex sonographic examination of the colon
The wall of the colon was scanned with a 7 MHz linear array transducer at the following locations: cecum, proximal and distal ascending colon, proximal and distal transverse colon, proximal and distal descending colon, sigmoid at the crossing point with the left psoas muscle, and rectum. Longitudinal and transverse sections of the colon were recorded and a short (less than 3 seconds duration) color duplex sonographic video was recorded under strictly standardized conditions [4]. An optimized preset of the ultrasound machine was consistently used including fixed color Doppler frequency, transducer type, gain, color scale, as well as other parameters of the manufacturer's imaging preset panel. All examinations were carried out with an Acuson Sequoia 512 ultrasound system (Acuson, Mountainview, California, USA).

Five colon segments were separately investigated: colon ascendens, transversum, descendens, sigmoideum and rectum. In 17 colonoscopies, 85 segments were involved. From these segments, 850 videos were recorded. These videos were examined to calculate 240 mean values of perfusion intensity according to their recording site and to correlate them with the respective biopsy site. These 240 perfusion values were then correlated to the 114 histologic specimens.

DTPM
DTPM was performed using the above-mentioned standardized color Doppler videos. These videos were sent via a computer network to a personal computer equipped with PixelFlux software [24] for DTPM. Each video was checked to discard those with movement artifacts. Distance and velocity calibration were carried out automatically by the software. The bowel wall was circumscribed to define the region of interest. The heart beat triggered a calculation of perfusion, which was performed automatically by the software. An example of two differently affected colon segments in a patient with UC is provided in Fig. 1.

The principle of DTPM refers the evaluation of tissue blood flow to all basic parameters relevant to

Table 1. Histological score parameters for correlation to perfusion measurements [9–23]*

Score points	0	1	2	3
Changes in crypt architecture [9]	None	Minor	Moderate	Severe
Depletion of goblet cells [10–12]	None	Minor	Moderate	Severe
Paneth cells distal to the left colon flexure [9]	None	Few	Some	Many
Lymphocytes [9,13,14]	Normal concentration	Minor increase	Moderate increase	Severe increase
Plasma cells [9,12]	Normal concentration	Minor increase	Moderate increase	Severe increase
Eosinophils [13,15]	Normal concentration	Minor increase	Moderate increase	Severe increase
Unspecific inflammatory infiltrates [9]	None	Minor	Moderate	Severe
PMNs in lamina propria and lamina epithelialis [16–18]	Normal concentration	Minor increase	Moderate increase	Severe increase
Crypt abscesses [9,11,12,17]	None	Few	Some	Many
Edema [11]	None	Minor	Moderate	Severe
Erosions or ulcerations [9,11]	None	Erosion	Large erosions	Ulceration
Regenerative epithelium [12,19,20]	None	Scarce	Significant	–
Fibrosis [15,21]	None	Minor	Moderate	Severe
Increased cryptal distance to muscularis mucosae [12,22,23]	None	Minor	Moderate	Severe

These histological data were correlated to the perfusion intensity of the bowel wall at the site of biopsy. PMN = polymorphonuclear leukocytes.

perfusion intensity. These parameters are the total area of all perfused vessels (arterial as well as venous vessels) in a tissue section and the mean perfusion velocity of all erythrocytes in such a section. Neither parameters are evaluated individually but with respect to the entire region of interest, i.e. the parameters are evaluated with respect to the whole area of tissue under investigation. Another important aspect of DTPM is its reference to complete heart cycles. All parameters are recorded for full heart cycles so that perfusion intensity is a rhythmic measure of tissue blood flow referring to the basic rhythm of one heart beat. This is necessary since perfusion greatly changes across the heart cycle, and this change may be more or less profound depending on the inflammatory activity.

Color Doppler ultrasound provides the necessary information to calculate the intensity of blood streaming through a given tissue plane. Perfusion intensity is calculated from the perfused area (A), the area of the region of interest (A_{ROI}) encompassing nonperfused parts of the tissue and perfusion velocity (*v*). It is defined as follows:

$$I = v \times A/A_{ROI} \ [\mathrm{cm/s} \times \mathrm{cm^2/cm^2} = \mathrm{cm/s}]$$

Standardization of image acquisition and selection of the region of interest is a necessary precondition to achieve reliable measurements. This is routine in daily practice because for a given organ (e.g. colon), sonographers use predefined ultrasound machine settings to achieve an optimal image quality. Basic settings kept constant were color Doppler frequency and gain.

Histology
A set of histological parameters (Table 1 [9–23]) was routinely applied during the pathological workup
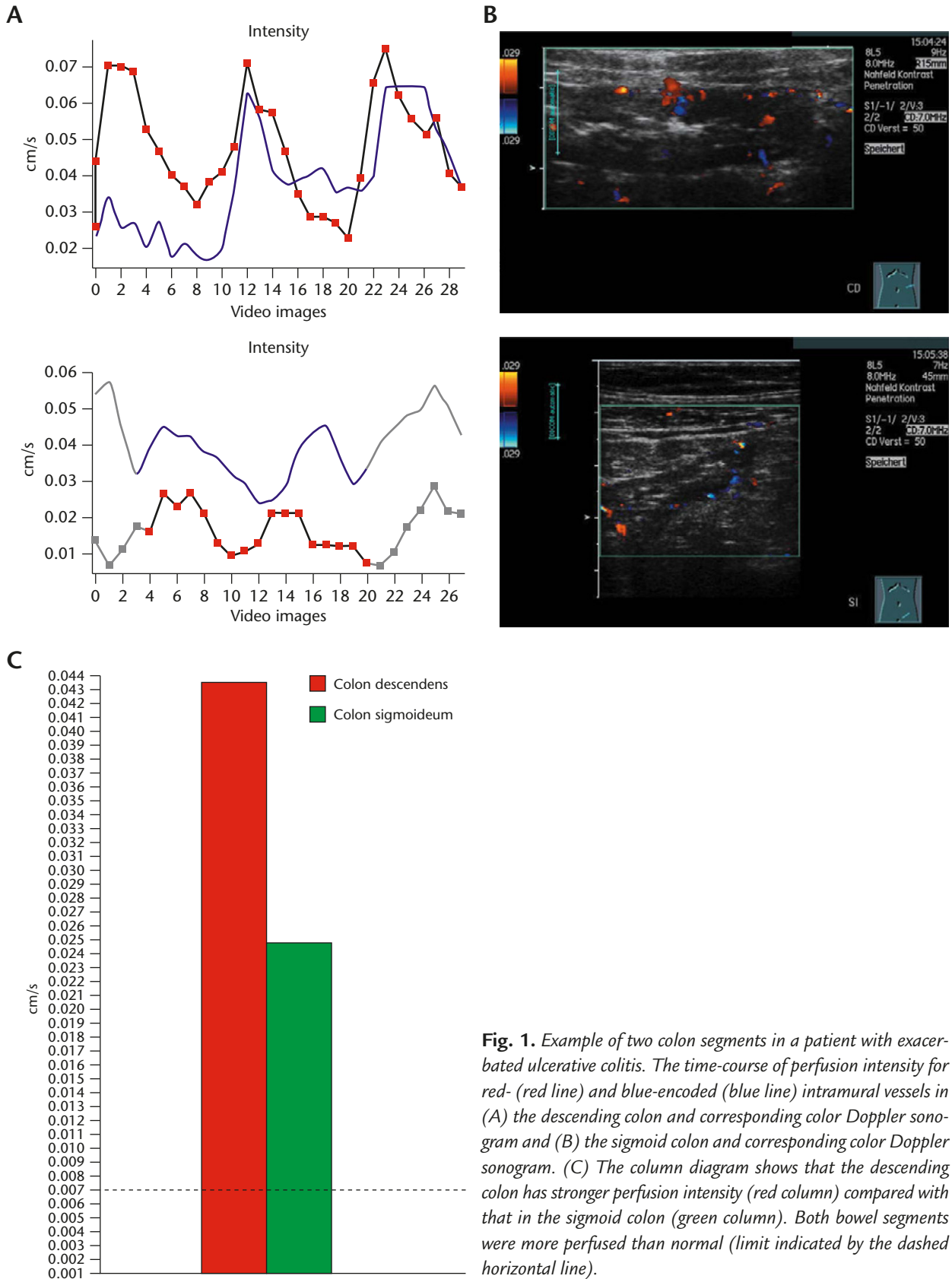
A

B

C

Fig. 1. *Example of two colon segments in a patient with exacerbated ulcerative colitis. The time-course of perfusion intensity for red- (red line) and blue-encoded (blue line) intramural vessels in (A) the descending colon and corresponding color Doppler sonogram and (B) the sigmoid colon and corresponding color Doppler sonogram. (C) The column diagram shows that the descending colon has stronger perfusion intensity (red column) compared with that in the sigmoid colon (green column). Both bowel segments were more perfused than normal (limit indicated by the dashed horizontal line).*

of colon biopsy specimens [17]. These parameters were assigned score points depending on the degree of their appearance.

Statistical analysis

Perfusion intensities were compared among score groups for all criteria applied by means of the Mann-Whitney *U* test. A *p* value less than 0.05 was regarded as significant for differences among the groups. The numbers of measurements displayed in the figures differ since groups with insufficient numbers were excluded from analysis.

Results

Several of the parameters tested were associated with a significant correlation of wall perfusion at the site of biopsy (see Figs. for *p* values). Neutrophils (Fig. 2), lymphocytic invasion (Fig. 3) of the wall and crypt abscesses were directly correlated (Fig. 4), whereas wall edema (Fig. 5) was inversely correlated with wall perfusion. On average, 10 video clips per colon segment were recorded. The average coefficient of variation of all perfusion intensity measurements was 0.530 (0.498–0.583).
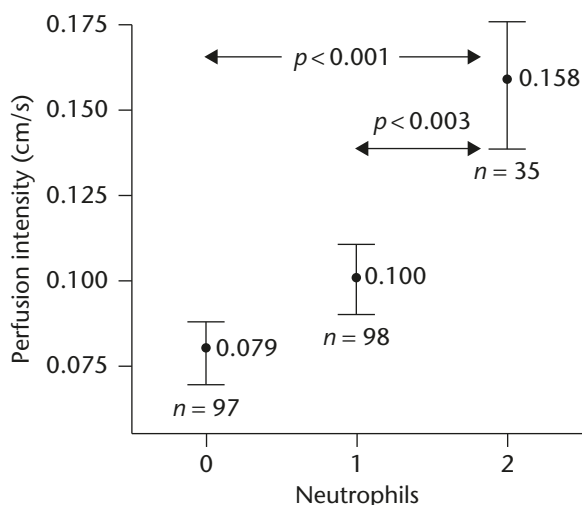
Discussion

Prominent sonographic signs of UC are wall thickening and abundant coloration in color Doppler sonography. Wall thickening is easily found and reproducible. Unfortunately, it is not suitable for evaluating the phase of UC or to distinguish ischemic from inflammatory colitis [25,26]. Perfusion of the bowel wall changes rapidly in the course of UC, either spontaneously or induced by treatment [27].

Titration of pharmaceuticals needs to be guided by a parameter that closely reflects the course of the disease. Evaluation of perfusion is thus the Ariadne's thread to the correct diagnosis of differentiating several forms of colitis (ischemic, inflammatory and vasculitic) and to quantify the degree of inflammation. Therefore, color flow evaluation has a discriminating power that cannot be achieved with B-mode ultrasound alone [28].

Hyperperfusion is a general indication of inflammation in IBD [29]. Vascular density is also increased in both CD and UC [30] Moreover, vascular changes are tightly correlated with the disease process [30]. These findings have prompted researchers to measure the actual inflammatory activity in IBD. Since
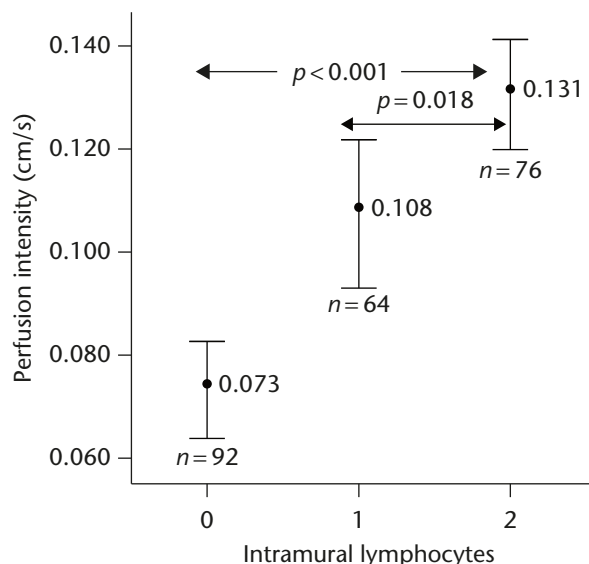

Fig. 2. *Significant colon wall perfusion differences were demonstrated between different histological score groups for neutrophil invasion of the bowel wall. n = number of perfusion measurements.*

Fig. 3. *Significant colon wall perfusion differences were demonstrated between different histological score groups for lymphocyte invasion of the bowel wall. n = number of perfusion measurements.*
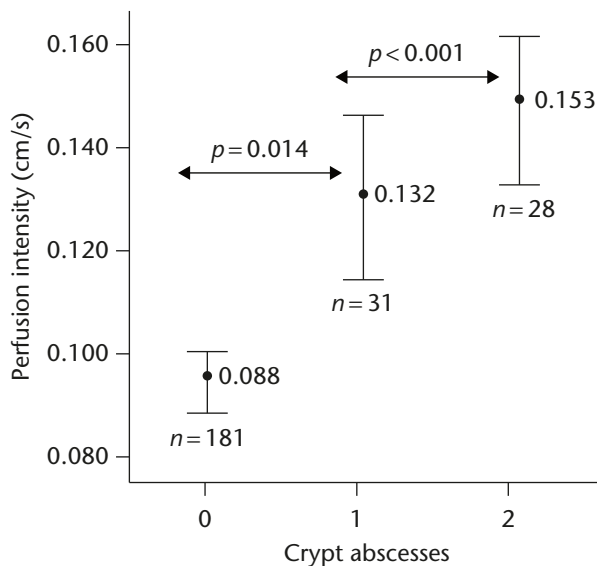
Fig. 4. *Significant colon wall perfusion differences were demonstrated between different histological score groups for crypt abscesses of the bowel wall. n = number of perfusion measurements.*

Fig. 5. *Reduction of bowel wall perfusion in cases with edema. n = number of perfusion measurements.*

direct estimation of the site of inflammation, which is the affected bowel segment, is difficult to obtain, indirect measures are used; indirect activity indices are proposed in several forms for CD and UC. These indices help to avoid endoscopies and have been shown to be closely correlated with invasive indices, which also take into account endoscopic criteria of inflammation [31]. However, even in the major forms of IBD, different modes of calculation from a variety of changing components have been proposed because of the lack of a satisfactory solution.

A method for direct and non-invasive evaluation of disease activity is required. Magnetic resonance imaging (MRI) provides better data on the degree of bowel inflammation than clinical scores of inflammation in an animal model of colitis [32]. However, ultrasound has many advantages over MRI such as higher spatial and time resolution, better availability and bedside use, lower costs and combination of imaging and palpation, and is therefore, the initial method of choice in suspected IBD [33]. This is why quantification of inflammatory activity by means of diverse sonographic approaches has been attempted.

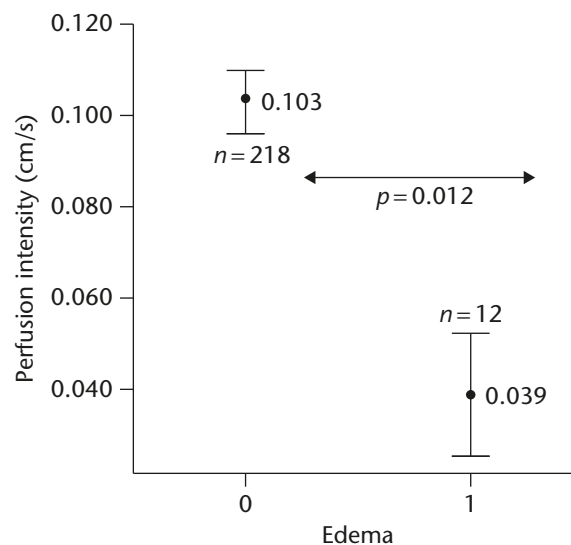Ultrasound was first proposed to observe changes in the perfusion pattern in the major arteries of the intestinal tract (mainly superior mesenteric artery). Correlation between the resistance index changes and disease activity in the downstream bowel segments has not been fully achieved [34]. This may be because severe inflammation in small intestinal segments might not be reflected in the main feeding artery, since the normal perfusion demand in the other non-affected segments have masked the effect. In this case, it is necessary to pinpoint the branch that solely feeds to the affected bowel segment. However, this has yet to be accomplished since the course of minor arteries inside the mesentery cannot be traced. One way to get around this problem is to directly estimate the hyperperfusion within the affected bowel segment [35,36]. This is rather simple, since the swollen and stiff segment can be easily retrieved by the sonographer, and thereby making reliable and repeatable examinations possible.

The first steps consisted of vessel counting in color Doppler sonographic images of the bowel wall [27,37]. A more recent approach is the use of sonographic contrast enhancers to study the increased contrast at the site of inflammation [38–41]. Contrast enhanced ultrasound and MRI of the intestines are significantly correlated in describing the activity of inflammation in CD [42]. Both approaches minimizes

invasiveness and rule out the masking effect of an evaluation that is too general for diseased as well as healthy bowel segments. Here we propose a similar but essentially different method: the dynamic measurement of perfusion from color Doppler sonographic videos. This method combines all the advantages of the previously mentioned local sonographic evaluation techniques while overcoming their limitations. The advantage over simple vessel counting is its refined technique. For instance, DTPM takes into account not only the number of vessels but also the velocity of each single pixel, i.e. the area occupied by vessels in relation to the entire cross-sectional area of the investigated bowel wall. All these data are calculated for an entire heart cycle, thus realistically reflecting the often prominent changes of perfusion intensity during one full heart beat. This method unites many of the demands made in an ideal method to quantify the intestinal perfusion on site.

Since the color of a certain pixel defines its velocity vector (v) directed straight towards the transducer, the true velocity (v_t) of the erythrocyte is given by the formula "$v = v_t / \cos \alpha$" if the angle α between the vessel and the ultrasound propagation line exists. In a healthy bowel segment, there is a regular pattern of microvessels with mucosal branches forming arcades, but this regularity is lost in IBD. In IBD, a distorted, irregular, interwoven and felt-like vessel organization may be found [43]. The chaotic distribution of small intraparietal vessels causes a constant error that has to be accepted. This does not hamper reliable perfusion measurements of the intestinal wall since the systematic error due to the individual angles of each intraparietal vessel is negligible. DTPM is not possible in videos with movement artifacts. In IBD, the bowel is thickened and shows only limited peristalsis. Therefore, it is not difficult to localize affected bowel segments reliably and to collect data from videos free of movement artifacts. In CD, there is a correlation between intestinal wall perfusion measurements by DTPM and disease activity [4].

We compared the DTPM results with scores from histological criteria of acute and chronic inflammation of the respective biopsy site. The reliability of histological evaluation of IBD has been demonstrated in several investigations [44–46]. Inter- and intra-observer concordance of histological evaluation of colitis subtypes has been established [47].

Score parameters from our study are in accordance with criteria for UC inflammation grading from the literature (Table 1 [9–23]) [22,23,48]. Significant differences could be demonstrated between different score groups among all criteria applied. Therefore, we conclude that DTPM is a helpful adjunct to the existing diagnostic methods in UC. It describes intestinal wall perfusion non-invasively and in a novel way, not only encompassing flow velocity changes of a single vessel—as the resistance index does—and delivering a surrogate as contrast enhancing (which also requires an invasive technique), but for the first time, calculating perfusion intensity from all relevant raw data as follows: the region of interest-related perfused area, and the mean flow velocity of all blood cells with respect to their changes during complete cardiac cycles. Thus, DTPM offers a tool to non-invasively follow the actual inflammatory activity, which in turn could help adapt treatment strategy to the actual need. Such a tailored therapy would avoid side effects and increase efficacy of treatment by decreasing the likelihood of over- as well as under-treatment. Overall costs of care of IBD patients may also be reduced. Further studies are necessary to determine the full potential of this technique in IBD, which has already been successfully applied in nephrology, urology and oncology.

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