Non-invasive longitudinal study of an MRI biomarker for the quantification of colon inflammation in a mouse model of colitis

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Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract which can strongly negatively affect the quality of life of patients suffering from this pathology [1]. Colonoscopy, the current gold-standard for IBD diagnostics and follow-up, is known to cause discomfort in patients. In addition, this technique doesn't permit to detect extracolonic lesions, being restricted to the diagnostics of visible changes in the mucosal surface. In this context, MRI can play a major role being a non-invasive imaging technique, characterized by good soft tissue contrast, high spatial resolution, and absence of ionizing radiation. We propose here an *in vivo* MRI longitudinal study of colon wall thickness as an imaging biomarker to detect and stage the severity of this disease in a mouse model of dextran sulfate sodium (DSS)-induced colitis. The results were validated against colonoscopy and standard *ex vivo* histology. The intra- and inter- operator reproducibility was assessed for all the measurements.

Material and methods: Study protocol: Female C57BL/6NCrl mice (n=26, 12 week-old, 21.7 ± 0.6 g) were used in the experiment. At day 0, 2% or 3% DSS (MP Biomedicals, Germany) was added to the drinking water of mice (n=10/group) for 5 days. Six mice were left as controls and no DSS was added to their tap water. Animals were imaged with colonoscopy and MRI on days 7, 11 and 21 to study the colitis progression. After the acquisition of the last images, animals were sacrificed and colons were extracted to perform histology. Body weights and drinking pattern were recorded daily. MRI Protocol: Images were acquired with a 7 T Biospec spectrometer (Bruker, Ettlingen, Germany), using a transmitter/receiver quadrature coil of 38 mm inner diameter. A soft hollow tube was gently inserted into the anus of the anesthetized mice. A small amount of water was flushed into the colon to get rid of residual stools. For each animal 25 consecutive axial slices of the colon of 1 mm thickness were acquired. The acquisition was performed using a 2D RARE sequence (TR = 3000 ms, TE = 36 ms, field of view 2.56x2.56 cm², matrix 256x256, 4 averages), for a total acquisition time of about 6 minutes. MR image analysis: Wall thickness was measured on three different points of the same axial slice (*Fig. 1g*) and was repeated on two different axial slices (roughly 1.2 and 1.8 cm from the anus). The wall thickness for each animal was computed as the average of the measured values. Residual water was easily identified because of its hyperintense MR signal and was thus excluded from the segmentation process. Colonoscopy: Images were acquired using a mini-endoscopic system (Coloview, Karl Storz, Tuttlingen, Germany) and evaluated according to the scoring system proposed in Ref. [2] on a scale from 0 to 15. Statistics: Data between different groups were compared using Kruskal-Wallis test with Dunn's multiple comparison test (non-parametric ANOVA) with a 0.05 significance level. Inter- and intra-observer reproducibility was evaluated using Bland-Altman analysis. Correlations were evaluated using Spearman coefficients.

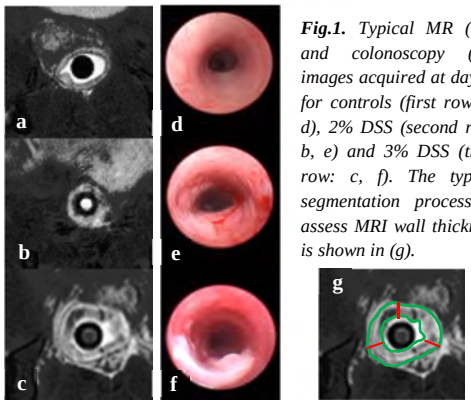

Fig.1. *Typical MR (a-c) and colonoscopy (d-f) images acquired at day 11 for controls (first row: a, d), 2% DSS (second row: b, e) and 3% DSS (third row: c, f). The typical segmentation process to assess MRI wall thickness is shown in (g).*

Results: Mice drinking water containing DSS showed a dose-dependent (p<0.01 starting from day 7) steady decrease of body weight up to day 8, followed by a slow recovery phase completed on day 21. A standard increase of body weight was observed in control mice throughout the 21 days. In *Fig. 1*, typical MR and colonoscopy images are shown for day 11. The measurement of the colon wall thickness by MRI (*Fig. 2a*) showed a significant difference between control and DSS-induced colitis mice at all the three time points. On day 11, a significant difference was observed in MRI wall thickness also between mice that received different concentrations of DSS (p=0.04). An excellent correlation and negligible bias were found for intra-observer (r= 0.924, p<0.0001, bias =0.01±0.03mm) and inter-observer measurements of MRI wall thickness (r=0.746, p<0.0001, bias =0.01±0.06mm, *Fig. 3*). Very similar results were achieved with the colitis score obtained from colonoscopy images (*Fig. 2b*). The mean endoscopic colitis score was significantly higher in DSS treated animals compared to control animals at the three time points. Thickening of the colon wall, changed vascular pattern and fibrin deposits were visible in the mucosa of DSS-induced colitis. Contrarily, the colon wall of healthy mice was characterized by a smooth translucent mucosa with normal blood vessel architecture showing no signs of colitis. The colonoscopy colitis score showed a significant difference between controls and DSS-induced colitis mice at all the three time points. On day 11, a significant difference was observed in colitis score also between mice that received different concentrations of DSS (p=0.0012). An excellent correlation and negligible bias were found for intra-observer (r= 0.943, p<0.0001, bias =0.2±1.1) and inter-observer measurements of colitis scores (r=0.943, p<0.0001, bias =0.2±1.1). Finally, an excellent correlation was obtained between the MRI wall thickness measurements and the colitis scores at all the three time points (r= 0.78, r =0.82, r=0.84 on days 7, 11 and 21 respectively with p<0.0001, *Fig. 4*) and with the wall thickness measured on histological slices at day 21 (r= 0.53, p= 0.019).

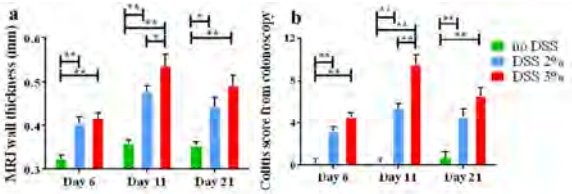

Fig. 2. *Bar plots showing (a) the wall thickness measured with MRI and (b) the colitis score obtained from colonoscopy. * = p<0.05, **= p<0.01.*

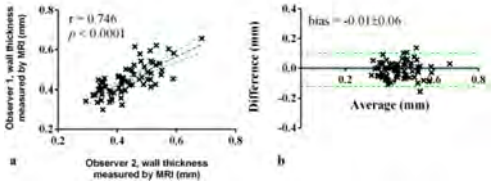

Fig.3. *Inter-observer reproducibility plots for the MRI wall thickness measurements. (a) Correlation between the measurements performed by two different observers and (b) Bland-Altman plot for the same data.*

Discussion: In this work, colon wall thickness measured by means of MRI was employed to differentiate mice with induced colitis from healthy animals while colonoscopy and standard histological analyses were used to validate the MRI results. The excellent correlation of the colitis score obtained from colonoscopy (the gold-standard to assess colon-related diseases) and the MRI wall thickness measurements *at all the time points* suggests that the segmentation procedure performed on RARE MR images is precise and accurate. Histology measurements performed at the endpoint confirmed the increase in the wall thickness of DSS-drinking animals as observed with MRI. All these findings suggest the feasibility of using non-invasive assessment of colon wall thickness as a robust non-specific biomarker for colon inflammation detection and follow-up. It is furthermore worthy to mention that at the second time point (highest inflammation time point) MRI wall thickness was significantly different between groups that received different amounts of DSS, suggesting that this biomarker is sensitive enough to stage different degrees of inflammation in the colon.

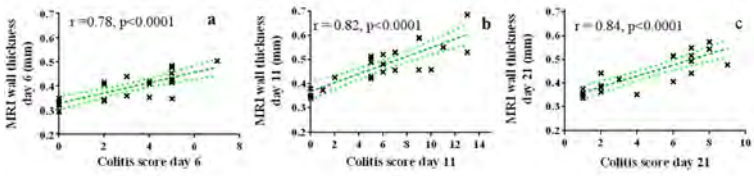

Fig.4. *Correlation plots between the wall thicknesses measured with MRI and the colitis score obtained from colonoscopy on days (a) 6, (b) 11, and (c) 21.*

Conclusion: In conclusion, the results presented in this work show that MRI is a powerful imaging tool able to *non-invasively* detect, quantify and longitudinally monitor the development of colitis. The data presented, validated with high-resolution colonoscopy imaging and conventional histopathologic analysis, clearly show the potential of MRI in *in vivo* pre-clinical longitudinal studies, including testing of new drugs or investigation of IBD development mechanisms. To our knowledge, this is the first study which proves the feasibility of a completely non-invasive *reproducible* longitudinal MRI quantitative detection of colitis *without the use of contrast agents*. The absence of ionizing radiation and high-resolution of MRI, along with the complete non-invasive nature and good reproducibility of the proposed protocol, make this imaging tool ideal for direct *translational applications.*

References: [1] *N Engl J Med*, 1991, 325(13):928-37 [2] *Nat Protoc*, 2006;1(6):2900-4