Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker in collagenous colitis (CC)

P099

Gunnar Andreas Walaas[1], Ingunn Bakke[1,4], Atle van Beelen Granlund[1,3], Celia Escudero-Hernândez[5], Torunn Bruland[1], Elin Synnøve Røyset[1,4], Silje Thorsvik[1,2], Andreas Münch[5,6], Ann Elisabet Østvik[1,2], Arne Kristian Sandvik[1,2,3]

[1]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU – Norwegian University of Science and Technology, Trondheim, Norway.
[2]Department of Gastroenterology, Clinic of Medicine, St. Olav`s University Hospital, Trondheim, Norway.
[3]Centre of Molecular Inflammation Research, NTNU – Norwegian University of Science and Technology, Trondheim, Norway.
[4]Department of Pathology, Clinic of Laboratory Medicine, St. Olav`s University Hospital, Trondheim, Norway.
[5]Department of Biomedical and Clinical Sciences (BKV), Linköping University, Linköping, Sweden.
[6]Division of Gastroenterology and Hepatology, Department of Biomedical and Clinical Sciences (BKV), Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden

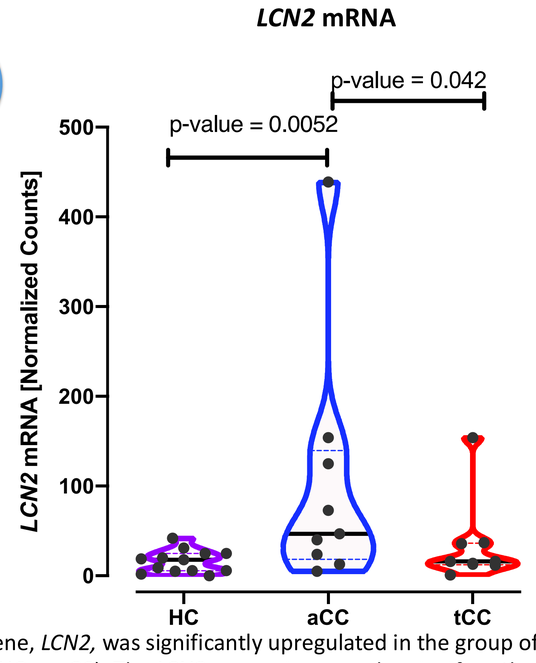
Background:

Neutrophil gelatinase-associated lipocalin (NGAL) is upregulated in intestinal epithelium in the inflammatory bowel diseases ulcerative colitis and Crohn`s disease and is a faecal biomarker with sensitivity and specificity comparable to calprotectin. Microscopic colitis (MC) is a common cause of chronic, watery diarrhoea and represents an inflammatory bowel disease with unknown etiology and pathogenesis. Diagnosis depends on histological evaluation of colonic biopsies. There is a need for new diagnostic tools, and this study examines the potential of NGAL as a biomarker in collagenous colitis (CC) as one of the two main histological forms of MC.

Method:

- Colonic biopsies were sampled at the Gastrointestinal Endoscopy Units at University of Linköping, Sweden and St. Olav's University Hospital in Trondheim, Norway. The biopsies were stored in Allprotect Tissue Reagent at -80℃ or fixed in 4% buffered formaldehyde.
- RNA sequencing was done on colonic biopsies from healthy controls (HC) and paired colonic biopsies from CC patients with active disease before treatment (aCC) and during response to budesonide (tCC), using SENSE total RNASeq library prep kit and RiboCop rRNA depletion on an Illumina Next Seq500 instrument.
- Faecal samples from the same patient groups and patients diagnosed with irritable bowel disease diarrhoea syndrome (IBS-D) were assayed for NGAL and Calprotectin (CalPro) by ELISA.
- Immunohistochemical staining and *in situ* hybridization was done to localize NGAL positive cells in an extended material of colonic biopsies, including the patients from the RNAseq and ELISA assays.

1



The NGAL gene, *LCN2,* was significantly upregulated in the group of active CC *vs*. HC. (log2 2.7, HC *vs* aCC). The *LCN2* expression was also significantly reduced during treatment (log2 -1.5, tCC *vs* aCC). Box and violin plot with individual values expresses distribution frequencies, median and interquartile range.

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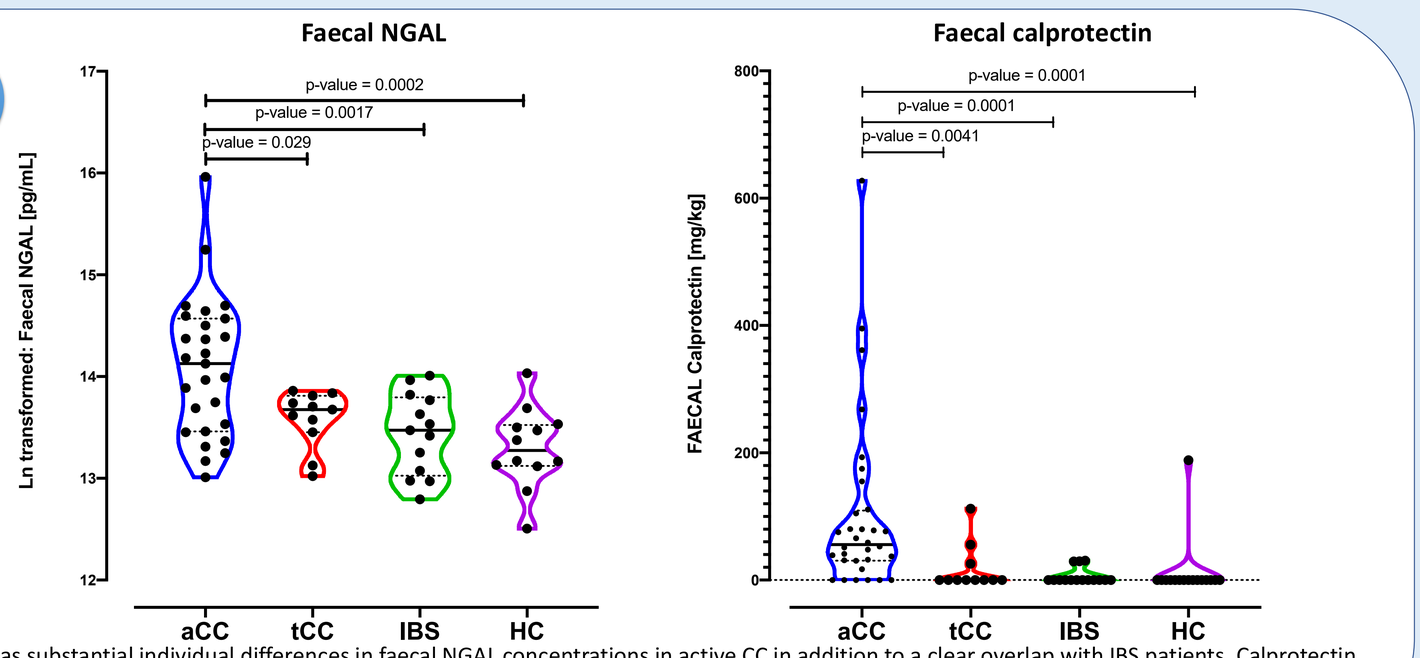
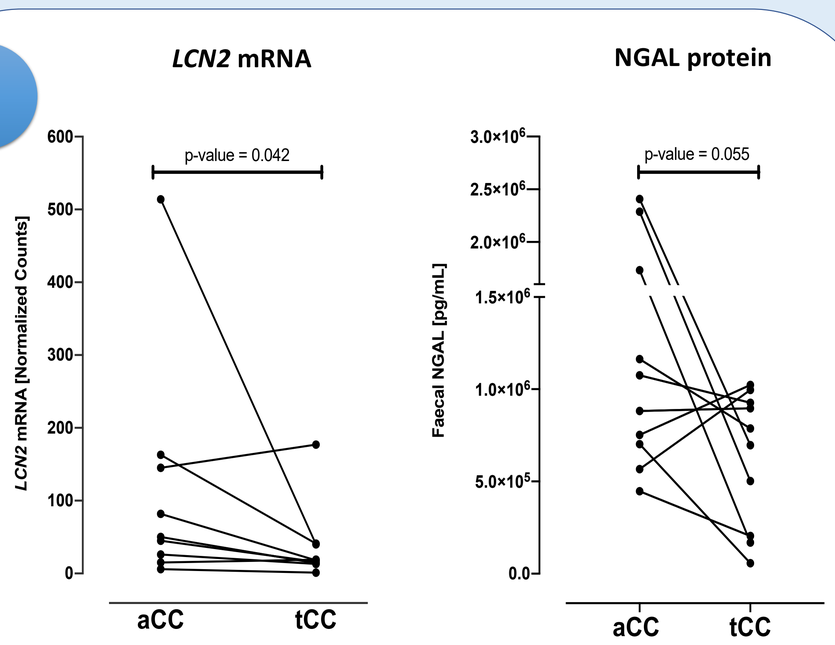


There was substantial individual differences in faecal NGAL concentrations in active CC in addition to a clear overlap with IBS patients. Calprotectin concentrations showed great variability within active CC. Faecal calprotectin levels showed stronger statistical difference between the groups, however most calprotectin values were below the clinical threshold for intervention (150 mg/kg). Box and violin plot with individual values expresses distribution frequencies, median and interquartile range.

3



In paired analysis of biopsies from active CC vs. budesonide-responsive CC in the same patients, *LCN*2 was significantly downregulated after treatment. Similarly, faecal NGAL concentrations declined after treatment. Pairs of individual values, before and after treatment, are shown.

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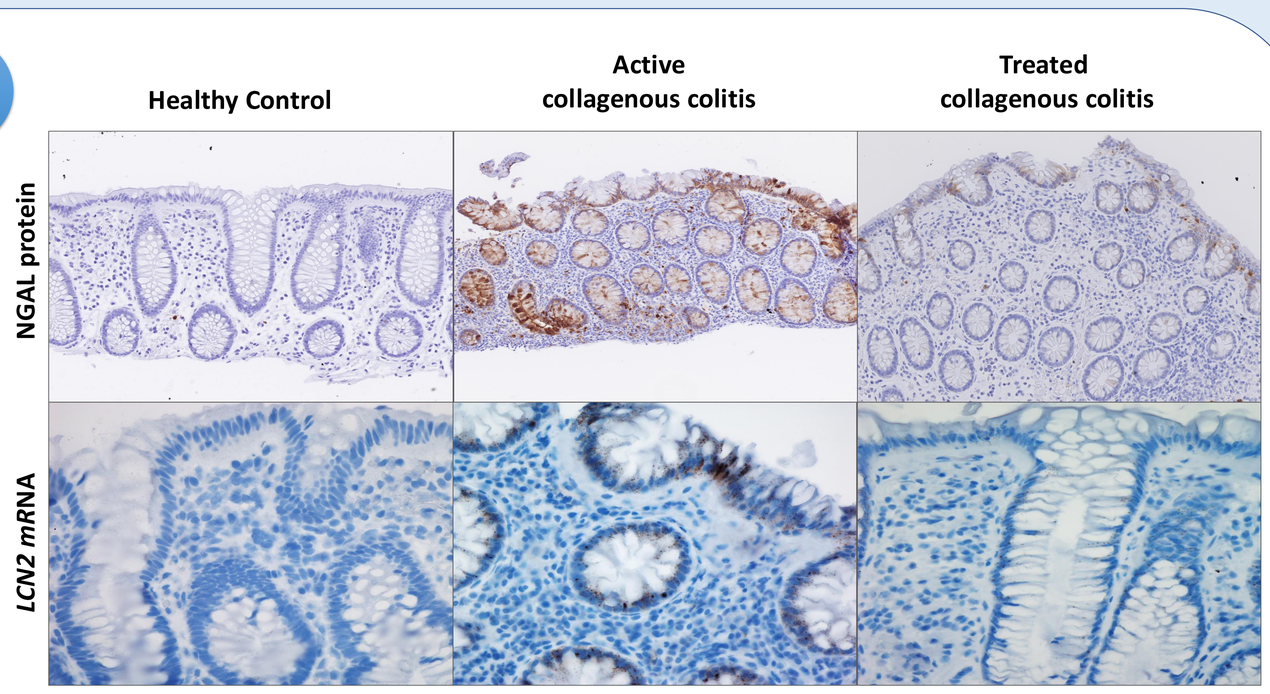

Both expression of NGAL protein and *LCN2* mRNA were clearly increased in the colonic epithelium of active CC compared to an almost absent expression in HC, and reduced to a scarce expression again during budesonide treatment.

Conclusions:

- The NGAL gene, *LCN2,* is upregulated in colonic epithelium of active CC vs. treated CC and healthy controls.
- Increased epithelial expression of NGAL in aCC is reflected by increase in faecal concentrations.
- NGAL is a promising biomarker in histologic evaluation of inflammatory activity related to CC and a potential faecal biomarker discriminating aCC from IBS-D.

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