IMMUNE-CELL SPECIFIC BIOMARKER OF EARLY INTESTINAL INFLAMMATION: NEUTROPHIL ELASTASE DEGRADED FRAGMENT OF TYPE III COLLAGEN IS ELEVATED IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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1) BACKGROUND

- Inflammatory Bowel Disease (IBD) is characterized by epithelial barrier injury of the gastrointestinal (GI) tract.
- The disease course encompasses abnormal immune responses and excessive secretion of proteases from immune cells. Neutrophils are the first to migrate into the inflamed interstitial matrix, where type III collagen is significantly deposited.
- Detection of mucosal inflammation in early stages is crucial to prevent cumulative clinical damage, as a delayed diagnosis can hinder effective treatment.

2) METHODS

- A competitive enzyme-linked immunosorbent assay targeting a human **neutrophil elastase derived fragment** of type III collagen was developed (**C3-HNE**).
- The **validation** was conducted by assessing its dilution recovery, matrix spiking, interference, and inter- and intra-assay variation.
- The **biological relevance** was evaluated in **commercial samples**, one **preclinical**, and two **clinical** studies. In the preclinical study, C3-HNE was measured in serum samples from a dextran sodium **sulfate (DSS)-induced colitis rat model**. In the commercial samples and clinical cohorts, C3-HNE was measured in serum from patients with IBD and healthy donors (HD).
- An *ex vivo* neutrophil model was optimized to assess the C3-HNE assay. **Primary neutrophils** (100.000 cells/well in 96 well-plate) were seeded in type III coated (78 µg/cm^2) wells. Activation media (IMDM with **LPS** 25 or 100 ng/ml) was added to the wells and the cells were incubated for 6 hours. Inactivated neutrophils were included as control.

3) AIM

The aim was to develop a biomarker that reflects early intestinal inflammation prior to it becoming medically evident; allowing us to distinguish patients that would benefit from an anti-inflammatory treatment.

4) RESULTS

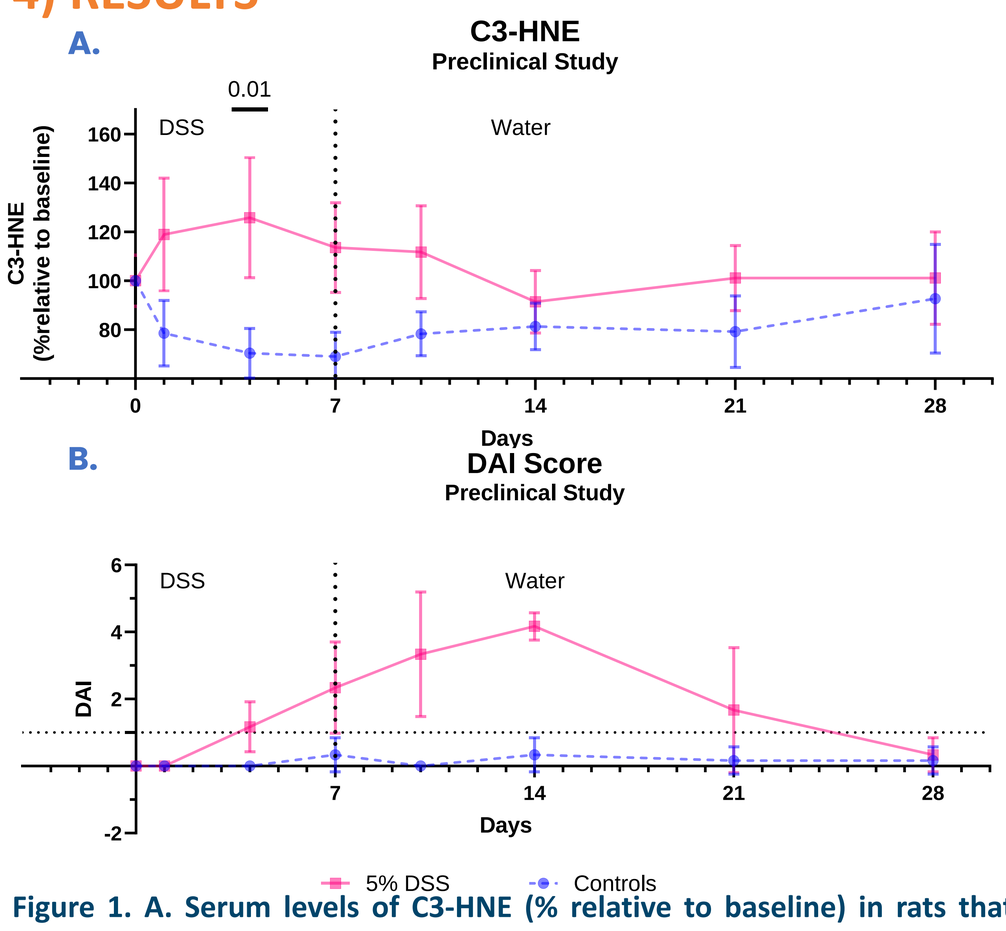

Figure 1. A. Serum levels of C3-HNE (% relative to baseline) in rats that were treated with 5% DSS (pink) and regular water (blue). B. The DAI score was monitored daily in rats that were treated with 5% DSS and regular water.

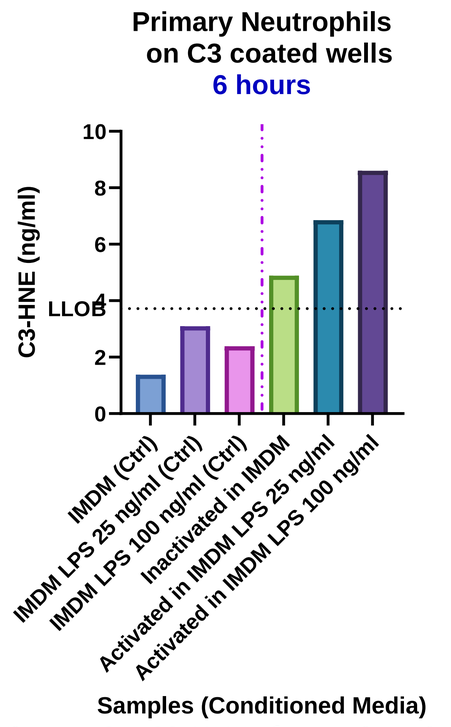

Figure 2. C3-HNE formation in conditioned media of primary neutrophils that were seeded in coated with type III collagen wells. Neutrophils were either inactivated or activated (LPS 25 ng/ml or 100 ng/ml) for 6 hours.

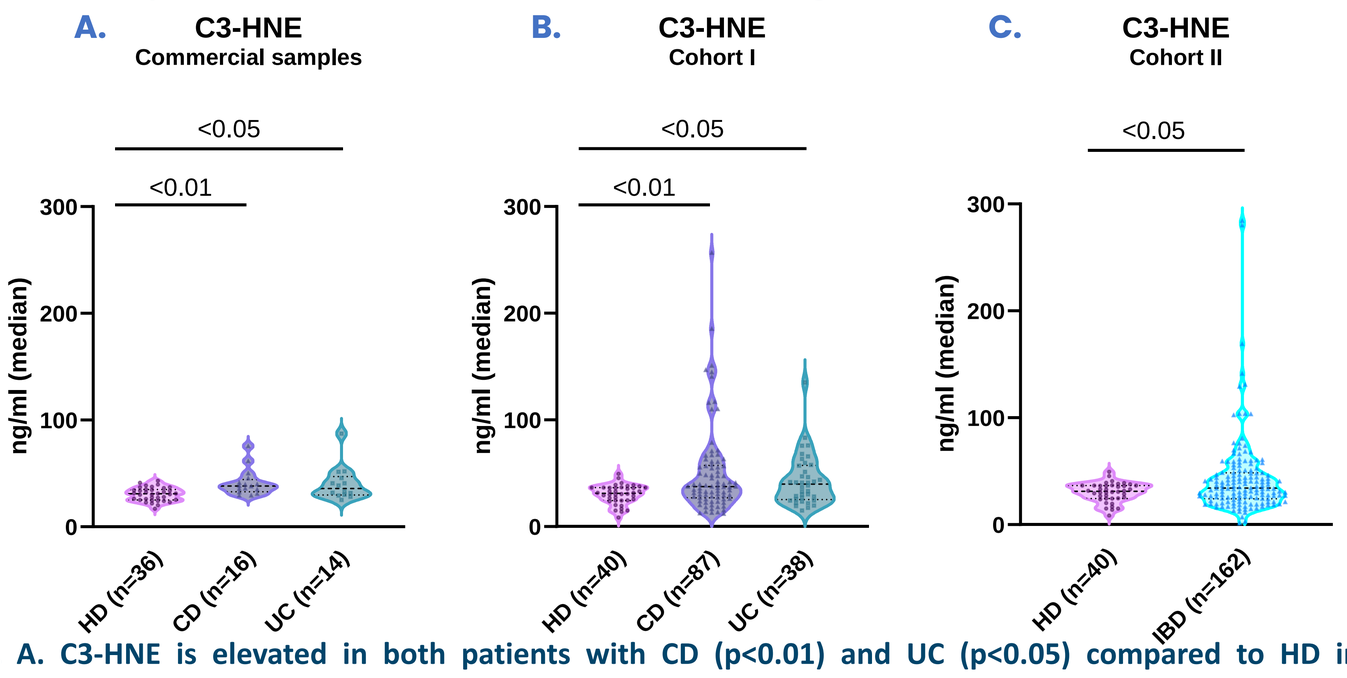

Figure 3. A. C3-HNE is elevated in both patients with CD (p<0.01) and UC (p<0.05) compared to HD in commercial samples. B. C3-HNE is elevated in both patients with CD (p<0.01) and UC (p<0.05) compared to HD in cohort I. C. C3-HNE is elevated in patients with IBD (p<0.05) compared to HD in cohort II.

ASSAY PARAMETER		RESULT		
Measurement range (LLOQ-ULOQ)		15.62 – 965.59 ng/ml		
Mean IC50		58.11 ng/ml		
Mean Slope		1.08		
		RECOVERY		
Intra-assay variation (CV%)		4 – 9%		
Inter-assay variation (CV%)		3 – 12%		
Dilution recovery in serum (1+3)		91 – 108%		
Freeze-thaw stability (5 cycles)		87 – 107%		
Kit Stability 24 hours at 20°C		99 – 119%		
Interference hemoglobin, low/high[a]		94 – 96%/ 87 – 98%		
Interference lipid, low/high[b]		96 – 105%/ 94 – 104%		
Interference biotin[c]		84 – 107%		
C3-HNE	**AUC [95% CV]**	**Sensitivity (%)**	**Specificity (%)**	**p-value**
Commercial Samples				
HD vs. CD	0.80 [0.67-0.92]	56.25	88.89	<0.001
HD vs. UC	0.72 [0.56-0.88]	50.00	88.89	=0.01
Cohort I				
HD vs. CD	0.66 [0.57-0.75]	47.06	92.50	<0.01
HD vs. UC	0.66 [0.52-0.79]	50.00	95.00	=0.01
Cohort II				
HD vs IBD	0.61 [0.52-0.69]	43.95	92.50	<0.05

[a]2.5/5 mg/ml, [b]1.5/5 mg/ml, [c]5/100 ng/ml

Table 1. Technical performance characteristics of the assay C3-HNE and ROC curve analysis results depicting the discriminative ability of C3-HNE between healthy and diseased (UC, CD and IBD) donors. The acceptance criteria for the validation were 100 ± 20 % and <15% for inter-intra variation.

Patient demographics

Cohort I

	UC	CD
Patients, n	38	87
Female, n (%)	20	33
Male, n (%)	18	54
Age, years (range)	39 [17, 62]	34 [18, 66]
BMI, kg/m² (range)	23.5 [15.4 - 39.6]	24 [15.5 - 40.4]
CRP, mg/L (range)	10.7 [0, 98.5]	8.5 [0, 172]
Fecal Calprotectin, µg/g (range)	354.6 [42.6, 810.7]	320.8 [75.9, 1714.7]
Montreal B, n (%)		
Luminal	NA	24 (29%)
Stricturing	NA	29 (35%)
Penetrating	NA	18 (22%)
Mix	NA	11 (14%)
Partial Mayo score, n (%)		
Remission	16 (43%)	NA
Mild	10 (27%)	NA
Moderate	6 (16%)	NA
Severe	5 (14%)	NA

Cohort II

	IBD
Patients, n	162
Responders, n (%)	147 (90.7%)
Nonresponders, n (%)	15 (9.3%)

Responders to Infliximab were defined as having a score of 0 according to the Physicians Global Assessment determined at Visit 3.

Additional patient demographical data is pending.

5) CONCLUSION

- C3-HNE is elevated in patients with IBD compared with HD.
- C3-HNE levels were elevated in conditioned media from primary neutrophils activated with LPS for 6 hours.
- C3-HNE reflects early stages of clinically apparent mucosal damage during experimental colitis.
- This biomarker holds the potential to identify early mucosal damage or acute inflammation in the GI tract; nevertheless, additional studies are needed to evaluate its clinical validity.

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