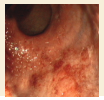
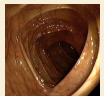
FECAL LACTOFERRIN, CALPROTECTIN, PMN-ELASTASE, CRP AND WHITE BLOOD COUNT AS INDICATORS FOR MUCOSAL HEALING AND CLINICAL COURSE IN ULCERATIVE COLITIS: A PROSPECTIVE 12-MONTH MONITORING STUDY

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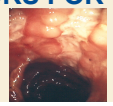

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INTRODUCTION

Ulcerative colitis (UC), characterized by periods of active disease and remission, is challenging to manage. Recent studies have identified mucosal healing as an optimal patient outcome.

AIMS

In this study, we investigated whether blood and fecal biomarkers of inflammation are able

To distinguish between mucosal healing defined by endoscopy from intestinal inflammation,

To differentiate between clinical remission and sustained clinical remission and

To show predictive value for a flare if levels at baseline are elevated.

METHODS

Endoscopy (Index EI - Rachmilewitz <=1 indicating mucosal healing) (baseline, 12 month), clinical activity index (CAI - Rachmilewitz), fecal Lactoferrin (FLA; cut-off: >=7.25µg/g), Calprotectin (CAL;>50µg/g) and PMN-Elastase (PMN-e;>0.062µg/g) (IBD-SCAN from Techlab, Blacksburg, USA for lactoferrin, ELISA-Kits from Immundiagnostik, Bensheim, Germany for calprotectin and PMN-elastase), serum CRP (≥0.5mg/dl) and white blood count (WBC>8.5/nl) (baseline, 1, 3, 6, 9, 12 month) were determined repeatedly and in events of acute flares.

Clinical status was **defined by the CAI** as follows: Patients in an acute clinical flare (CAI > 4), in clinical remission (CAI≥2 and ≤4) and in sustained clinical remission (CAI < 2, normal bowel frequency and no blood in stool).

Inflammatory status was **defined by the CAI and endoscopy** as follows: Patients in an acute clinical flare and endoscopic intestinal inflammation, patients in clinical remission and endoscopic intestinal inflammation, Patients in clinical remission and mucosal healing.

RESULTS

Table 1: Median, range and p-values of the five diagnostic biomarkers as **defined by the CAI.**

Diagnostic tool median ± STD	Acute clinical flare CAI>4 N = 52	clinical remission CAI≤2 and ≤4 N = 119	sustained clinical remission CAI<2 N = 358	p-value from Mann Whitney U
Lactoferrin	33.1 (0.1 – 145.0)	20.0 (0.1 – 167.6)		0.109
	33.1 (0.1 – 145.0)		3.6 (0.0 – 160.7)	*< 0.000*
		20.0 (0.1 – 167.6)	3.6 (0.0 – 160.7)	*< 0.000*
Calprotectin	25.0 (1.7 – 105.6)	19.2 (0.01 – 365.5)		0.057
	25.0 (1.7 – 105.6)		9.2 (0.01 – 369.3)	*< 0.000*
		19.2 (0.01 – 365.5)	9.2 (0.01 – 369.3)	*0.004*
PMN-elastase	0.06 (0.0 – 0.4)	0.04 (0.0 – 0.4)		*0.034*
	0.06 (0.0 – 0.4)		0.02 (0.0 – 0.7)	*< 0.000*
		0.04 (0.0 – 0.4)	0.02 (0.0 – 0.7)	*0.022*
CRP	0.5 (0.1 – 10.6)	0.2 (0.1 – 9.9)		0.051
	0.5 (0.1 – 10.6)		0.2 (0.0 – 3.0)	*< 0.000*
		0.2 (0.1 – 9.9)	0.2 (0.0 – 3.0)	*0.004*
White blood count	7.3 (3.0 – 14.7)	6.6 (2.7 – 13.7)		*0.011*
	7.3 (3.0 – 14.7)		6.3 (3.1 – 14.9)	*< 0.000*
		6.6 (2.7 – 13.7)	6.3 (3.1 – 14.9)	0.054

Table 2: Median levels and p-values of the five diagnostic biomarkers according **as defined by the CAI and endoscopy.**

Diagnostic tool median (range)	clinically active intestinal inflammation N = 35	in clinical remission intestinal inflammation N = 37	Mucosal healing N = 107	p-value from Mann Whitney U test
Lactoferrin	43.7 (0.1 – 145.0)	36.7 (0.2 – 160.7)		0.687
	43.7 (0.1 – 145.0)		4.4 (0.0 – 126.9)	*< 0.000*
		36.7 (0.2 – 160.7)	4.4 (0.0 – 126.9)	*< 0.000*
Calprotectin	25.0 (1.7 – 105.6)	19.8 (1.4 – 98.5)		0.292
	25.0 (1.7 – 105.6)		10.4 (0.01 – 62.1)	*< 0.000*
		19.8 (1.4 – 98.5)	10.4 (0.01 – 62.1)	*0.003*
PMN-elastase	0.06 (0.0 – 0.4)	0.03 (0.0 – 0.4)		0.052
	0.06 (0.0 – 0.4)		0.02 (0.0 – 0.7)	*< 0.000*
		0.03 (0.0 – 0.4)	0.02 (0.0 – 0.7)	*< 0.011*
CRP	0.7 (0.1 – 10.6)	0.2 (0.2 – 9.9)		*0.011*
	0.7 (0.1 – 10.6)		0.2 (0.0 – 2.8)	*< 0.000*
		0.2 (0.2 – 9.9)	0.2 (0.0 – 2.8)	0.243
White blood count	7.0 (3.0 – 14.7)	6.5 (3.7 – 13.0)		0.098
	7.0 (3.0 – 14.7)		6.4 (3.8 – 13.0)	*0.036*
		6.5 (3.7 – 13.0)	6.4 (3.8 – 13.0)	0.793

Table 3: Sensitivity and specificity, PPV and NPV for the five diagnostic biomarkers compared to sustained clinical remission **(CAI < 2, normal bowel frequency and no blood in stool) as gold standard**

Diagnostic tool		sensitivity in %	specificity in %	PPV in %	NPV in %
Lactoferrin	n = 515	63	63	54	70
Calprotectin 50	n = 515	8	98	75	60
PMN-elastase	n = 515	34	86	63	64
CRP	n = 526	32	87	66	83
white blood count	n = 529	23	90	63	61

Table 4: Sensitivity and specificity, PPV and NPV for the five diagnostic biomarkers compared to mucosal healing using **endoscopy as gold standard**

Diagnostic tool		sensitivity in %	specificity in %	PPV in %	NPV in %
Lactoferrin	n = 174	75	63	57	80
Calprotectin 50	n = 174	10	99	88	63
PMN-elastase	n = 174	32	87	61	66
CRP	n = 176	46	83	64	70
white blood count	n = 179	23	89	57	64

Table 5: Sensitivity and specificity, area under the curve and p- value for ROC analyses, relative risk with elevated biomarkers at baseline to develop a flare within the study.

Diagnostic tool		Cut-off	AUC (95% CI)	sensitivity in %	specificity in %	Diagnostic accuracy in %	p-value	RR (95% CI), p-value
Lactoferrin	n = 161	11.9	0.734 (0.654 – 0.813)	70.3	70.1	70.2	*< 0.000*	*1.99 (1.47 – 2.71), p<0.001*
Calprotectin 50	n = 161	13.9	0.700 0.619 – 0.782)	64.1	63.9	64.0	*< 0.000*	*1.58 (1.20 – 2.09), p=0.001*
PMN-elastase	n = 163	0.035	0.697 (0.614 – 0.780)	54.7	73.2	64.0	*< 0.000*	*1.67 (1.21 – 2.29) p<0.001*
CRP	n = 151	0.25	0.651 (0.562 – 0.740	62.1	62.9	62.5	*0.001*	*1.52 (1.15 – 2.0) p=0.002*
White blood count	n = 166	n.s.	0.569 (0.477 – 0.660)	n.s	n.s		0.133	

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SUMMARY OF RESULTS

In 91 patients (45 female, mean age 52±13.4 years), 620 CAI and 179 endoscopies were performed.

A total of 42 (46%) patients developed a clinical flare.

Results for **Clinical status** as **defined by the CAI** are shown in table 1 and 3.

Results for **Inflammatory status** as **defined by the CAI and endoscopy** are shown in table 2 and table 4.

Using pre-defined cut-offs, only increased levels for FLA at baseline were associated with a significant higher risk of flaring (RR 1.69, p=0.018).

Using optimized cut-offs (for FLA were 11.9µg/g, CAL 13.9µg/g, PME-e 0.035µg/g and CRP 0.25mg/dl), patients with elevated FLA at baseline had a relative risk (RR) of 1.99 (95% CI 1.47-2.71, p<0.000) to develop a flare, CAL RR=1.58, (1.20–2.09, p<0.000) PMN-e RR=1.67 (1.21–2.29, p<0.000), CRP: RR=1.52 (1.15–2.0, p<0.001) (WBC: n.s.) as shown in table 5.

CONCLUSIONS

Fecal biomarkers have the potential to distinguish between mucosal healing and intestinal inflammation and to differentiate between active clinical flare and sustained clinical remission. Using pre-defined cut-offs, only fecal lactoferrin had predictive potential. Using optimized cut-offs, FLA, Cal, PMN-e and CRP were predictive of a flare.

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