BIOMARKERS AS PREDICTORS OF FLARES IN INFLAMMATORY BOWEL DISEASE

M. Lavric [1,], S. de Roock[2], N. Ryter[3], M. Brückner[1,4], J. Däbritz[1,5], T. Jermann[3], B. Prakken[2], D. Foell[1]*

[1]Department of Paediatric Rheumatology and Immunology, University Hospital Muenster, Muenster, Germany, [2]Laboratory of Translational Immunology & Division of Pediatrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands, [3]Bühlmann Laboratories AG, Schönenbuch, Switzerland, [4]University Department of Medicine B, University Hospital Münster, [5]University Children's Hospital Rostock, Ernst-Heydemann-Str. 8, 18057, Rostock, Germany

Background

Inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) are chronic-remittent inflammatory disorders of the gastrointestinal tract still evoking challenging clinical diagnostic and therapeutic situations. As both CD and UC are characterized by unpredictable episodes of relapse and remission, the main treatment goal is to induce and maintain remission. In a prospective multicenter study pediatric and adult patients with CD and UC in remission (remission and active disease were defined by clinical disease activity scores) were consecutively recruited and followed up between April 2008 and June 2011 in 4 independent German outpatient clinics specialized in IBD, collecting blood and stool samples.[1,2] Studies on stool samples showed the utility of S100A12 and S100A8/A9 to predict IBD flares.[1,2] The purpose of the shown analyses was to also analyze blood-based biomarkers for the prediction of unstable remission, i.e. to identify patients who may need an intensified maintenance therapy.

Cohort

In a multi-platform/multi-centre approach to identify serum biomarkers with the potential to predict unstable remission, eighty (80) paired (initial and follow-up) serum samples from 40 IBD patients were analyzed. Of these, 60 samples were from 30 patients diagnosed with UC and 20 from 10 patients diagnosed with CD. In the UC group, 30 samples were from 15 patients with stable remission (remission initial and follow-up) and 30 samples from 15 patients with unstable remission (initial remission, follow-up acute flare) of disease. Similarly, in the CD group, 10 samples were from 5 patients with stable remission (remission initial and follow-up) and 10 samples from 5 patients with unstable remission (initial remission, follow-up acute flare) of disease. S100A12 ELISA was performed at University Hospital Muenster, S100A8/A9 ELISA at Bühlmann Laboratories AG and a 50-plex Luminex assay was performed at University Medical Center Utrecht.

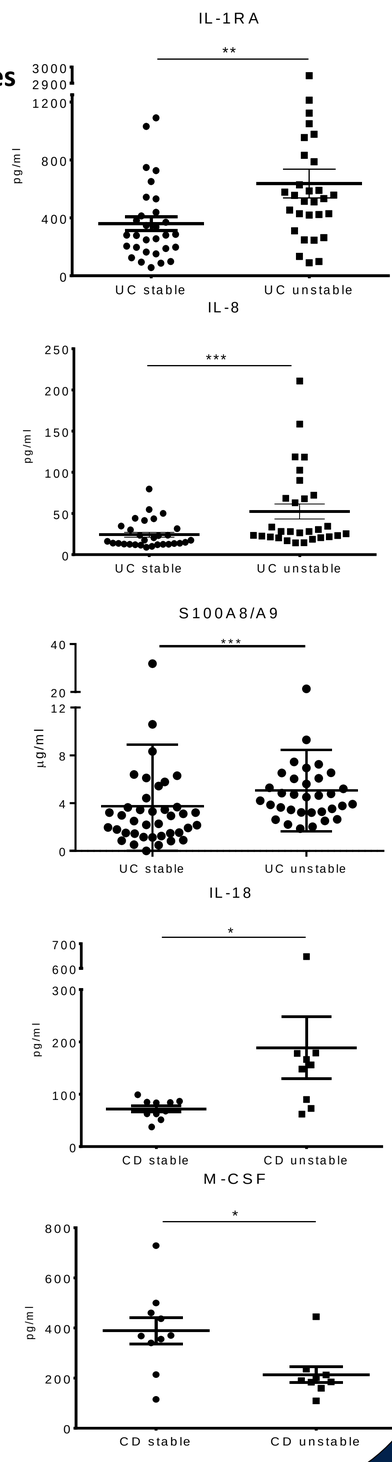
Results

Analyses showed that there are no differences when comparing values for the 50 analytes in paired initial and follow-up samples from either the stable or unstable remission groups. In contrast, the concentrations of 22 analytes (IFN α, β and γ; IL-1α, -1β, -1RA, -1R1, -8, -12p70, -13, -15, -21, -23p19 and -25; TNFβ and –R2; FGF basic, G-CSF, I-TAC, IP-10, MIG and Galectin-1) were significantly (Mann-Whitney U test, p<0,05) higher and the concentration of IL-18BPa was significantly lower in samples from UC patients experiencing unstable remission (either remission or flare), when compared to samples from UC patients with stable remission. In the CD group, the concentration of IL-18 was significantly higher and the concentrations of IL-31, MIP1β, M-CSF and FGF basic were significantly lower in samples from patients with unstable remission when compared to samples of patients with stable remission. S100A8/A9 concentrations were significantly higher in UC patient samples with unstable remission, when compared to samples of patients with stable remission.

Table/Heat Map: Higher (bold red, p<0,05) or lower (bold green, p<0,05) concentration of analyte in IBD unstable group than in stable group of serum samples
Light red - >1,5-fold increase, non significant (NS, p>0,05); Light green - < 1,5-fold decrease, NS; Grey - <1,5-fold increase or >1,5-fold decrease, NS
All analytes in pg/ml, except S100A12 (ng/ml) and S100A8/A9 (µg/ml).

Analytes	UC stable Mean ± SEM	UC unstable Mean ± SEM	P value	CD stable Mean ± SEM	CD unstable Mean ± SEM	P value
IL-1Ra	**360,1 ± 48,27**	**637,4 ± 99,41**	**0,0041**	614,5 ± 144,9	780,5 ± 286,4	0,7618
IL-1α	**4,996 ± 0,7967**	**15,99 ± 6,236**	**0,0106**	6,263 ± 1,121	10,11 ± 2,754	0,2428
IL-1β	**2,159 ± 0,3266**	**4,608 ± 0,7595**	**0,0249**	2,835 ± 0,6089	6,483 ± 2,432	0,3562
IL-2	12,41 ± 5,950	34,45 ± 16,36	0,4987	8,135 ± 3,828	7,713 ± 7,148	0,9212
IL-5	20,30 ± 9,604	20,88 ± 3,422	0,0834	17,01 ± 4,241	40,89 ± 19,26	0,6126
IL-6	11,10 ± 2,662	12,87 ± 4,100	0,1031	11,07 ± 2,710	6,771 ± 2,873	0,2698
IL-10	10,60 ± 6,798	5,124 ± 1,327	0,3542	2,993 ± 0,6070	2,692 ± 0,4675	0,6058
IL-12p70	**29,83 ± 5,067**	**304,0 ± 179,9**	**0,0046**	25,18 ± 5,399	21,38 ± 5,092	0,6965
IL-13	**8,561 ± 1,544**	**22,24 ± 4,525**	**0,0039**	29,25 ± 14,39	138,1 ± 88,18	0,8421
IL-15	**5,408 ± 0,4583**	**7,965 ± 0,7234**	**0,0037**	5,711 ± 1,522	12,79 ± 5,645	0,4967
IL-17	60,04 ± 16,30	156,1 ± 66,79	0,4510	59,32 ± 10,89	42,65 ± 23,38	0,0650
IL-18	113,3 ± 10,83	137,8 ± 7,517	0,0881	**71,99 ± 5,967**	**188,8 ± 59,30**	**0,0101**
IL-21	**1438 ± 263,0**	**4560 ± 884,0**	**0,0070**	4347 ± 2351	17080 ± 10650	0,7577
IL-22	121,9 ± 17,55	166,1 ± 23,26	0,1654	152,4 ± 38,40	106,6 ± 31,98	0,4894
IL-23p19	**204,3 ± 98,73**	**2617 ± 749,5**	**0,0003**	1040 ± 662,9	6471 ± 3660	0,0848
IL-25	**1380 ± 186,3**	**3605 ± 583,4**	**0,0068**	1347 ± 315,2	2474 ± 598,9	0,1220
IL-31	520,6 ± 225,0	2642 ± 1721	0,7849	**1503 ± 634,1**	**104,3 ± 46,41**	**0,0343**
IL-33	47,00 ± 20,33	214,0 ± 137,1	0,6115	116,7 ± 48,20	8,064 ± 2,458	0,0831
IL-37	1270 ± 344,9	3451 ± 1507	0,0675	1660 ± 301,1	1216 ± 601,0	0,3148
TNFα	0,1975 ± 0,06787	2,233 ± 0,7336	0,1144	2,373 ± 1,164	25,92 ± 6,115	0,1333
TNFβ	**7,080 ± 2,589**	**63,29 ± 17,10**	**0,0265**	55,61 ± 35,13	248,7 ± 158,0	0,8413
IFNα	**4,721 ± 2,282**	**13,54 ± 3,028**	**0,0184**	24,24 ± 12,44	81,41 ± 48,78	1,0000
IFNβ	**1209 ± 242,5**	**3926 ± 641,1**	**0,0002**	2423 ± 774,3	9527 ± 4969	0,2991
IFNγ	**5,297 ± 1,006**	**12,54 ± 2,053**	**0,0137**	14,62 ± 6,799	58,14 ± 33,16	0,2698
I-309	1,241 ± 0,2272	2,832 ± 1,534	0,9380	2,021 ± 0,6022	1,714 ± 0,5831	0,8421
MCP-1	99,32 ± 6,740	97,01 ± 5,406	0,9940	92,28 ± 13,04	76,46 ± 3,576	0,3562

Analytes	UC stable Mean ± SEM	UC unstable Mean ± SEM	P value	CD stable Mean ± SEM	CD unstable Mean ± SEM	P value
MIP1α	29,05 ± 2,313	41,13 ± 7,577	0,8458	39,41 ± 5,933	33,55 ± 8,173	0,3154
MIP1β	79,64 ± 5,281	89,39 ± 13,05	0,7617	**60,98 ± 7,539**	**42,76 ± 5,428**	**0,0435**
Eotaxin	82,67 ± 6,616	81,26 ± 3,992	0,6383	69,51 ± 12,02	81,62 ± 4,484	0,3562
TARC	115,4 ± 14,72	140,3 ± 19,19	0,2523	180,5 ± 32,82	136,4 ± 28,50	0,1333
MDC	771,2 ± 40,42	710,6 ± 53,88	0,2223	939,2 ± 130,4	818,6 ± 92,35	0,7802
TECK	602,7 ± 77,55	506,8 ± 53,17	0,3670	446,6 ± 99,73	439,8 ± 107,6	0,8421
IL-8	**24,22 ± 3,046**	**52,49 ± 9,027**	**0,0009**	83,42 ± 34,55	98,31 ± 56,05	0,6038
MIG	**43,58 ± 11,68**	**72,25 ± 8,661**	**0,0001**	54,29 ± 11,85	79,78 ± 28,08	1,0000
IP-10	**213,2 ± 16,73**	**369,8 ± 55,62**	**0,0054**	287,0 ± 43,86	266,3 ± 73,50	0,4470
I-TAC	**59,40 ± 8,940**	**113,7 ± 14,17**	**0,0037**	168,6 ± 19,99	141,8 ± 38,35	0,0947
BLC	58,94 ± 32,56	32,75 ± 3,714	0,1314	50,51 ± 24,27	117,0 ± 53,96	0,2428
G-CSF	**32,02 ± 11,88**	**105,6 ± 23,31**	**0,0002**	120,9 ± 66,84	255,4 ± 162,1	0,6965
M-CSF	276,6 ± 29,84	330,7 ± 32,93	0,2587	**388,8 ± 52,14**	**213,6 ± 31,21**	**0,0133**
FGF basic	**95,54 ± 17,70**	**180,9 ± 51,21**	**0,0218**	**146,8 ± 22,52**	**79,91 ± 25,73**	**0,0350**
Cathepsin B	10480 ± 803,0	11060 ± 949,7	0,4903	5756 ± 544,6	7034 ± 862,2	0,4002
IL-18Bpa	**1784 ± 132,3**	**1374 ± 98,42**	**0,0296**	1092 ± 142,5	1576 ± 369,1	0,6038
IL-1R1	**10,09 ± 1,206**	**23,53 ± 7,071**	**0,0052**	8,992 ± 0,7390	9,594 ± 1,120	0,4747
IL-1R2	5781 ± 516,6	5793 ± 406,4	0,5904	3375 ± 388,7	2813 ± 746,8	0,3154
TNF-R1	3100 ± 291,0	3413 ± 250,7	0,2955	2012 ± 266,4	2648 ± 458,8	0,2775
TNF-R2	**2328 ± 130,4**	**2754 ± 109,3**	**0,0121**	2071 ± 227,7	2509 ± 348,9	0,4002
sIL-2R	892,1 ± 400,9	462,2 ± 72,69	0,4646	206,1 ± 44,82	244,1 ± 54,30	0,5726
Galectin-1	**39510 ± 1770**	**58530 ± 8070**	**0,0030**	33890 ± 3999	36760 ± 7844	0,9682
Galectin-7	1886 ± 303,8	3605 ± 1461	0,8855	3425 ± 1074	1146 ± 102,1	0,3154
Galectin-9	7955 ± 519,9	9197 ± 1177	0,7849	9370 ± 2162	7163 ± 1031	0,8421
S100A12	122,1 ± 28,45	115,0 ± 17,03	0,3166	107,0 ± 22,93	107,4 ± 18,76	0,7234
S100A8/A9	**3,744 ± 0,8235**	**5,059 ± 0,5930**	**0,0004**	2,205 ± 0,4649	2,920 ± 0,4321	0,1483



Conclusions and perspectives

1. The multi-platform, multi-center analyses of IBD patient sera identified 23 molecular markers with the potential to predict disease flares in UC: IFN α, β and γ; IL-1α, -1β, -1RA, -1R1, -8, -12p70, -13, -15, -21, -23p19 and -25; TNFβ and –R2; FGF basic, G-CSF, I-TAC, IP-10, MIG and Galectin-1, S100A8/A9.
2. In addition, 5 molecular markers with the potential to predict disease flares in CD were identified: IL-18, IL-31, MIP1β, M-CSF and FGF basic.
3. In parallel, stool samples collected from the same patients are also being analyzed for IBD relapse biomarkers via a multi-platform/multi-centre approach for potential biomarkers, including miRNA biomarker analyses of stool and sera.
4. Furthermore, in view of the relatively small amount of patients included/sera analyzed, especially in the case of CD, analyses are planned on larger sample sizes and validation cohorts to validate the findings.

References

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CONTACT: Miha.Lavric@ukmuenster.de