

Interleukin-1 β , interleukin-6, and other novel inflammatory/immune biomarkers in advanced colorectal cancer.

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Background

- Advanced colorectal (CRC) is an aggressive malignancy with a 5-year survival rate of <7%.
- Inflammation plays a significant role in the pathogenesis of advanced colorectal cancer (CRC).
- Soluble inflammatory biomarkers can be secreted by cancer cells into the tumor microenvironment, and their roles remain unclear.
- To explore the possible contribution of this mechanism in promoting systemic immunosuppression, the pre-treatment levels of cytokines, chemokines and some growth factors were researched in advanced CRC patients.

Methods

- The study included 40 advanced CRC patients compared to 30 healthy controls.
- Using ELISA procedures, plasma levels of the cytokines (IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, TNF- α , IFN- γ), chemokines (eotaxin, IP-10, MCP-1, MIP-1 α , MIP-1 β), growth factors (FGF, G-CSF, GM-CSF, PDGF-bb, and VEGF), as wells as high-sensitivity C-reactive protein (hs-CRP), were measured to identify novel biomarkers in this disease. In addition, we recorded the inflammatory full blood count (FBC) composite ratios, including NLR and PLR.
- We compared plasma levels and possible associations of these biomarkers.

Statistical Analysis

- Receiver-operating characteristic (ROC) curve analysis was used to determine the optimal cut point for the inflammatory markers.
- Progression-free survival (PFS) was estimated using the Kaplan–Meier method.
- Statistical comparisons were made by using the log-rank test.
- The Mann-Whitney U-test was used to compare levels of the various test biomarkers between CRC patients and healthy controls.

Results

Table 1. Patient Demographics.

Demographics (n=40)	
Median Age = 67 years (range 57 to 84)	
Gender	
Male	23 (58%)
Female	17 (42%)
Site	
Left colon	23 (58%)
Right colon	4 (10%)
Rectum	13 (32%)
Metastatic sites	
1 metastatic site	21 (53%)
>1 metastatic site	19 (47%)
Line of treatment	
First line	20 (50%)
>1 line	20 (50%)
Mutations	
MMR	
Repair-proficient	24 (60%)
Repair-deficient	0
Unknown	16 (40%)
KRAS	
Wild type	7 (18%)
Mutated	9 (22%)
Unknown	24 (60%)
BRAF	
Wild type	15 (38%)
Mutated	1 (2%)
Unknown	24 (60%)
NRAS	
Wild type	15 (38%)
Mutated	1 (2%)
Unknown	24 (60%)

Figure 1. Kaplan Meier survival analysis showing time to progression (TTP).

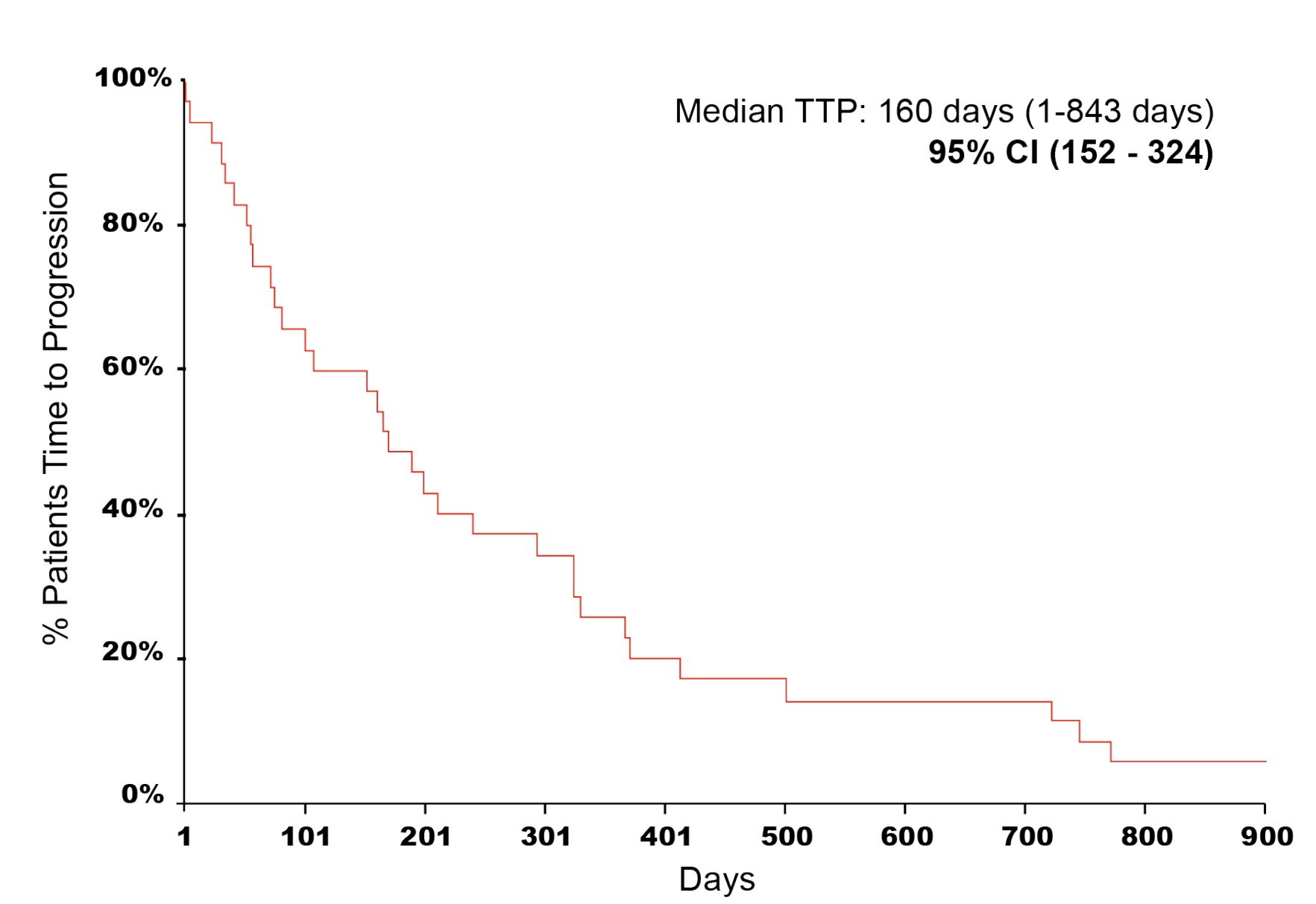


Table 2. Comparison between levels of biomarkers between CRC patients and healthy controls

		Median	95% CI	p-value
IL-1b	↑	2.03	1,2 - 2,63	0,0087
Control		1,07	0,77 - 1,41	
IL-1ra	↓	190,44	169,37 - 229,33	0,5529
Control		233,53	146,83 - 339,07	
IL-2	↓	0,25	0,25 - 0,79	0,0123
Control		1,63	1,63 - 1,67	
IL-4	↑	3,16	2,65 - 3,41	0,1457
Control		2,73	2,38 - 3,16	
IL-5	↑	5,91	0,93 - 14,32	0,388
Control		5,34	5,34 - 5,86	
IL-6	↑	2,1	0,38 - 3,58	0,0161
Control		0,49	0,14 - 0,77	
IL-8	↑	12,36	7,72 - 16,04	0,000
Control		3,45	2,29 - 5,77	
IL-9	↓	337,43	332,86 - 362,46	0,0936
Control		367,07	331,34 - 432,52	
IL-10	↑	1,97	1,29 - 3,93	0,000
Control		0,9	0,75 - 1,27	
IL-12	↑	15,26	11,91 - 21,74	0,000
Control		30,0	1,9 - 8,46	
IL-13	↑	6,98	5,11 - 8,32	0,000
Control		1,69	1,24 - 2,48	
IL-17	↑	21,45	19,23 - 23,11	0,000
Control		13,18	11,7 - 17,36	
Eotaxin	↑	60,0	52,02 - 62,29	0,0001
Control		38,8	28,88 - 48,9	
FGF basic		19,64	19,64 - 22,79	0,473
Control		19,86	16,15 - 22,74	
G-CSF	↑	154,16	133,54 - 172,00	0,000
Control		66,4	57,71 - 102,43	
GM-CSF	↑	3,04	2,67 - 3,74	0,000
Control		0,76	0,56 - 0,81	
IFN-g	↑	8,31	6,56 - 9,60	0,0357
Control		6,83	6,13 - 7,27	
IP-10	↑	388,35	295,58 - 456,83	0,0010
Control		216,73	183,63 - 266,06	
MCP-1(MCAF)	↑	18,8	16,47 - 23,30	0,0959
Control		16,37	13,82 - 20,61	
MIP-1a	↑	2,43	1,96 - 2,84	0,000
Control		1,29	1,02 - 1,51	
PDGF-bb	↑	459,57	312,53 - 537,97	0,0432
Control		369,91	135,14 - 454,78	
MIP-1b	↑	459,57	312,53 - 537,97	0,000
Control		154,4	120,36 - 171,94	
TNF-a	↑	134,5	119,94 - 140,67	0,000
Control		55,49	48,62 - 73,32	
VEGF	↓	9,1	9,1 - 59,70	0,1036
Control		12,94	9,1 - 16,75	
CRP	↑	4,22	2,9 - 7,58	0,0061
Control		1,59	0,42 - 3,37	

Table 3. Comparison between levels of biomarkers, between TTP $\geq$ 120 days and TTP <120 days, in CRC patients.

		TTP (days)	Median	95% CI	p-value
IL-1b	↑	< 120	2,28	0,55 - 3,80	0,9633
		$\geq$ 120	2,03	1,41 - 2,83	
IL-2		< 120	0,25	0,25 - 1,31	0,4681
		$\geq$ 120	0,25	0,25 - 1,31	
IL-6	↑	< 120	6,79	1,66 - 11,15	0,006
		$\geq$ 120	0,83	0,12 - 2,45	
IL-8	↑	< 120	21,52	7,72 - 39,56	0,037
		$\geq$ 120	9,11	5,13 - 15,78	
IL-10		< 120	1,97	1,27 - 6,43	0,6178
		$\geq$ 120	1,97	1,29 - 3,93	
IL-12	↑	< 120	18,5	11,91 - 21,74	0,5549
		$\geq$ 120	15,26	8,46 - 21,74	
IL-13	↓	< 120	6,98	3,04 - 9,82	0,8901
		$\geq$ 120	7,44	5,11 - 9,4	
IL-17	↑	< 120	21,45	16,99 - 24,75	0,8658
		$\geq$ 120	20,35	18,12 - 23,66	
Eotaxin	↑	< 120	60,32	44,73 - 65,98	0,8062
		$\geq$ 120	58,89	45,05 - 71,69	
G-CSF	↑	< 120	157,54	126,51 - 242,09	0,3905
		$\geq$ 120	142,78	126,51 - 185,13	
GM-CSF	↑	< 120	3,4	1,84 - 4,70	0,7691
		$\geq$ 120	2,67	1,84 - 3,74	
IP-10	↑	< 120	475,04	303,32 - 737,53	0,0705
		$\geq$ 120	361,13	226,75 - 456,83	
MIP-1a	↑	< 120	2,5	1,6 - 4,54	0,5806
		$\geq$ 120	2,21	1,79 - 2,96	
PDGF-bb	↑	< 120	504,86	302,43 - 663,83	0,2763
		$\geq$ 120	379,59	264,11 - 554,43	
MIP-1b	↑	< 120	110,15	99,53 - 114,83	0,2832
		$\geq$ 120	104,19	99,22 - 108,02	
TNF-a	↑	< 120	140,16	125,17 - 146,81	0,1672
		$\geq$ 120	132,43	115,74 - 140,67	
IFN-g	↑	< 120	9,39	6,56 - 14,27	0,0734
		$\geq$ 120	7,44	6,56 - 8,31	
CRP	↑	< 120	5,79	1,22 - 33,60	0,3000
		$\geq$ 120	3,53	2,51 - 5,83	

Table 4. No significant differences were detected in the inflammatory FBC composite ratios with the TTP cut-off point of 120 days.

		TTP (days)	Median	95% CI	p-value
SIRI*	↑	< 120	2,55	0,92 - 3,57	0,3116
		$\geq$ 120	1,79	0,74 - 2,28	
AISI	↑	< 120	587,73	261,13 - 966,86	0,2973
		$\geq$ 120	426,73	159,52 - 787,95	
PLR	↑	< 120	154	117 - 301	0,6256
		$\geq$ 120	142	97 - 173	
NLR	↑	< 120	3,26	1,78 - 5,65	0,1883
		$\geq$ 120	2,85	1,36 - 3,62	
MLR	↑	< 120	0,51	0,24 - 0,64	0,3418
		$\geq$ 120	0,34	0,25 - 0,42	

*SIRI: Systemic inflammation response index (neutrophil count $\times$ monocyte count) / lymphocyte count.
AISi: Aggregate index of systemic inflammation (Neutrophils $\times$ Platelets $\times$ Monocytes) / Lymphocytes.
NLR: neutrophil/lymphocyte ratio
MLR: monocyte/lymphocyte ratio.
PLR: Platelet/lymphocyte ratio.

Figure 2. Significant associations between inflammatory markers.

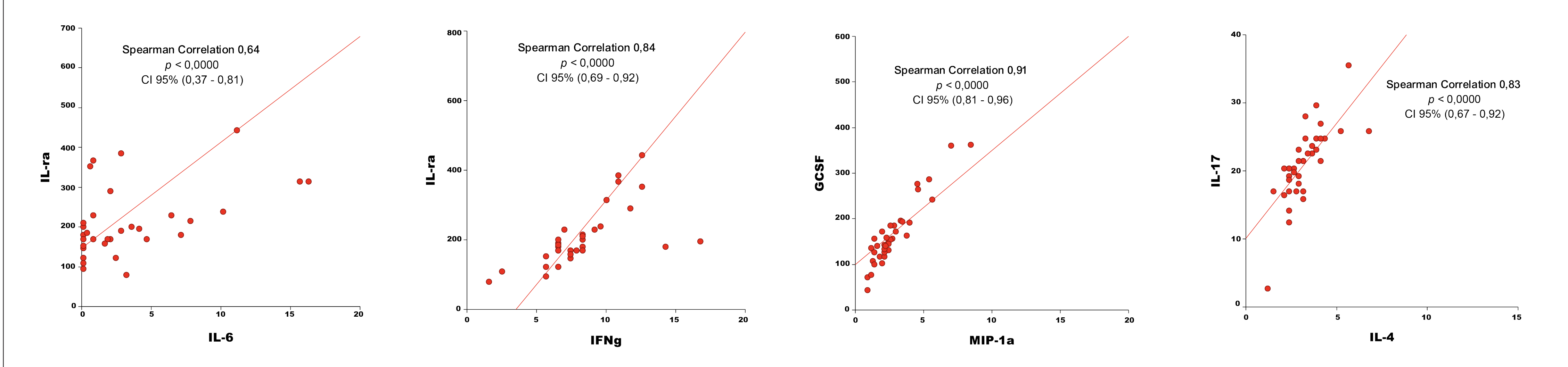


Table 5. ROC curve cut-off values and AUC (95% CI) for levels of biomarkers with a significant difference between patients and controls.

Criterion	AUC	P-Value	95% Confidence Limits
IL 1b	0,5381	0,3645	0,2897 - 0,7186
IL2	0,5778	0,1921	0,3767 - 0,7269
IL6	0,8016	0,0001	0,5767 - 0,9136
IL8	0,7492	0,0018	0,5292 - 0,8748
IL10	0,5603	0,2834	0,3207 - 0,7325
IL12	0,5619	0,2646	0,3398 - 0,7247
IL13	0,5159	0,4395	0,2844 - 0,6905
IL17	0,5508	0,3046	0,3275 - 0,7158
Eotaxin	0,4857	0,5572	0,2688 - 0,6557
GCSF	0,6222	0,1146	0,383 - 0,7833
GMCSF	0,5524	0,3117	0,3103 - 0,7272
IFNg	0,7175	0,0092	0,4851 - 0,8552
IP10	0,6635	0,0455	0,4304 - 0,8136
MIP1a	0,5889	0,1934	0,352 - 0,7549
PDGFbb	0,6048	0,1445	0,376 - 0,7641
MIP1b	0,6444	0,0764	0,4029 - 0,8021
TNFa	0,6762	0,0324	0,4442 - 0,8232
CRP	0,6016	0,1655	0,3581 - 0,7685

Inflammatory markers were tested for significance in relation to

- age (<60 years, $\geq$ 60 years),
- gender (male, female),
- the site of disease (left side, right side, rectum),
- metastatic sites (>1 metastatic site, 1 metastatic site),
- and the line of treatment (1st line, more than one line).

None of these variables showed a significant difference.

Figure 3. Heatmaps demonstrating the difference of inflammatory markers between patients and controls.

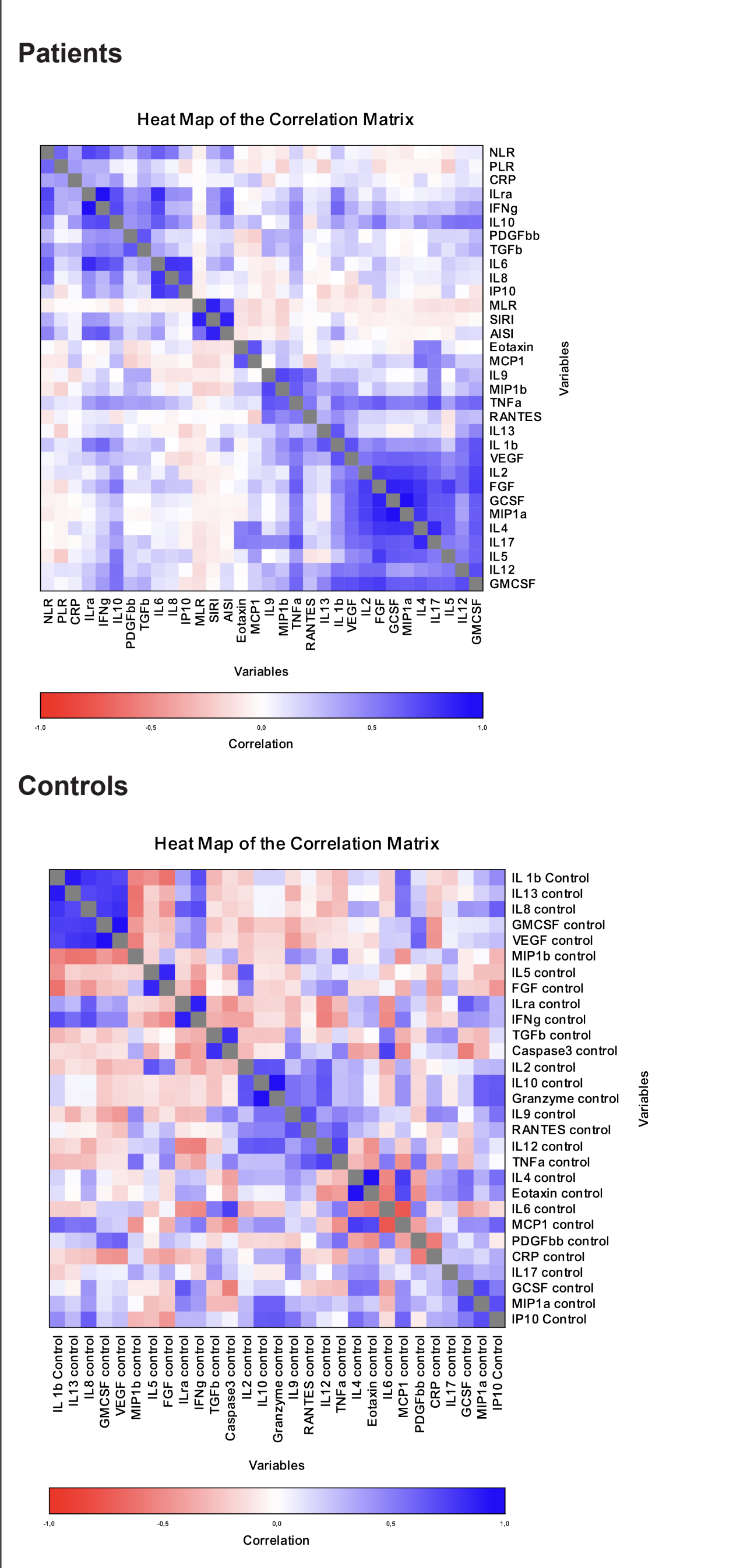


Table 6. Correlations between IL-1b, IL-1 α , IL-6, IL-8 IP10, MIP-1a and MIP-1b with other inflammatory markers.

Variables	IL 1b	ILRa	IL6	IL8	IP10	MIP1a	MIP1b
NLR	0.223919	0.726857	0.628809	0.525508	0.192457	-0.087861	-0.090886
MLR	-0.068813	-0.009102	-0.040938	-0.066898	-0.071522	-0.048352	-0.201141
SIRI	0.156559	0.403746	0.289277	0.106516	0.032593	-0.052016	-0.183424
AISI	0.378766	0.617766	0.384634	0.057529	-0.012751	-0.045268	-0.127271
PLR	0.083791	0.307128	0.135583	0.040783	-0.126421	-0.037119	-0.188459
IL 1b	1	0.50524	0.25128	0.000594	-0.084426	0.388792	0.430284
ILRa	0.50524	1	0.831072	0.436726	0.414723	0.009893	0.163248
IL2	0.419994	0.039483	0.050917	0.21292	-0.094786	0.745891	0.067334
IL4	0.410348	0.055059	0.036759	0.063543	-0.046232	0.792372	0.253657
IL5	0.195291	0.111698	0.189558	0.157726	0.199569	0.633094	0.082244
IL6	0.25128	0.831072	1	0.799645	0.781628	0.05292	0.035562
IL8	0.000594	0.436726	0.799645	1	0.722409	0.025787	-0.071338
IL9	0.253698	0.065744	-0.085642	-0.117343	-0.10898	0.003978	0.724944
IL10	0.371841	0.686025	0.644398	0.502073	0.36588	0.207497	0.083453
IL12	0.541345	0.209423	0.137377	0.103762	0.006067	0.358737	0.228155
IL13	0.669771	0.110946	-0.033418	-0.028509	-0.19198	-0.041689	0.355787
IL17	0.372107	0.074325	0.044441	0.068307	-0.044093	0.636984	0.46691
Eotaxin	-0.038104	0.001307	0.020412	0.106788	0.075026	-0.059299	0.238105
FGF	0.491882	0.200964	0.267913	0.216285	0.214071	0.853579	0.241609
GCSF	0.392198	0.047999	0.105477	0.079347	0.054186	0.997035	0.134173
GMCSF	0.675574	0.228585	0.110874	0.08944	-0.108847	0.631913	0.324451
IFNg	0.595464	0.953247	0.710674	0.287552	0.263731	0.142148	0.221203
IP10	-0.084426	0.414723	0.781628	0.722409	1	0.010187	0.040279
MCP1	0.071114	-0.040621	-0.059021	-0.05834	-0.019641	0.141917	0.244799
MIP1a	0.388792	0.009893	0.05292	0.025787	0.010187	1	0.12266
PDGFbb	0.146313	0.397825	0.264938	0.121695	0.13366	-0.020098	0.29476
MIP1b	0.430284	0.163248	0.035562	-0.071338	0.040279	0.122661	1
RANTES	0.432198	0.075403	0.002574	-0.040636	-0.004136	0.124195	0.452899
TNFa	0.589072	0.370521	0.33465	0.257488	0.162846	0.489571	0.636887
VEGF	0.682632	0.201283	0.080606	-0.127476	-0.165575	0.621014	0.381166
CRP	0.102164	0.336979	0.204019	0.119624	0.018427	-0.030856	0.020601
TGFb	0.162617	0.452383	0.349954	0.254404	0.104398	-0.032406	0.224103