Immune marker changes and risk of multiple myeloma: a nested case-control study using repeated pre-diagnostic blood samples

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Supplemental Data

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Supplementary Methods

Immune marker measurement

Based on an estimated medium effect size (Cohen's d = 0.5) power analyses indicated a power above 90% to replicate previous findings[1] assuming 65 matched case-control pairs, two tails, and a significance level of 0.05 (Gpower 3.1). Immune markers were measured by a Luminex bead-based multiplex assay from Millipore (USA). Both pre-diagnostic baseline and repeated samples from each study participant were analyzed. Samples from matched cases and controls were included in random order in the same analytical batch to minimize the influence of potential batch-effects on downstream analyses. All samples (N = 260) were measured in duplicate and quality control samples were included on every plate. Intra-assay coefficients of variation were: 8.4% for MCP-3, 9.1% for MIP-1α, 12.4% for MIP-1β, 8.7% for TGF-α, 8.4% for VEGF, 9.8% for FGF-2, 10.2% for fractalkine, 12.6% for IL-13, 8.0% for TNF-α, and 9.3% for IL-10.

M-protein assessment

M-protein assessment was performed in both baseline and repeated blood samples of 61 future myeloma cases ($N_{Samples}$ = 122). Protein- and immunofixation electrophoresis were applied to detect M-proteins in 122 samples (Sebia, France). A Cobas 8000 analyzer (Roche Diagnostics, Germany) was used to measure the total immunoglobulin (Ig) amount of IgG, IgA, and IgM with kits from the same manufacturer and kappa-lambda free light chains (FLCs) with the Freelite® assay (The Binding Site Group Ltd, UK) among these samples. All analyses were performed by experienced lab technicians. Results were evaluated blinded with respect to the chronological order of the samples. M-proteins of IgG type were quantified by means of visual inspection, while M-proteins of IgA (N = 8) and IgM (N = 1) type were quantified by the total protein amount of the affected Ig. For definitions of an abnormal FLC ratio and immunoparesis we considered reference values of the hospital's laboratory; abnormal FLC ratio (<0.44 or >1.99), reference values for Igs were; IgA (0.9 – 4.5 g/L); IgG (6.7 – 14.5 g/L); and IgM (0.3 – 2.1 g/L).

Statistical analyses

Missing values for measurements below the limit of quantification (N = 89; 3.4% of all data points), were multiply imputed after log transformation.[2] The imputation model was based on log transformed values for all immune markers, case-control status, and analysis plate.[3] Extreme concentration data of all markers were winsorized according to the 1st and 99th percentile to reduce their influence. In all statistical analyses body mass index (BMI) and smoking status were included as covariates. Missing values for BMI (N = 13) and smoking status (N = 2) at one of the blood draws, were replaced by the corresponding value from the other sampling time point from the same individual.

The applied linear mixed model included case-control status, the interaction term between case-control status and time, BMI, and smoking status as fixed effects. Time represented the duration between sample collection and case diagnosis, i.e. set to zero at the date of the case diagnosis. Intercepts for each individual and matched case-control pairs were included as random effects.

To facilitate comparison with a previous study based on single samples,[1] immune marker levels found to be significantly different among cases as compared to controls in linear mixed modeling were also analyzed using logistic regression. Concentration data were split into four categories, using the quartiles among controls at baseline as cut-off for baseline and repeated measures. Analyses of the entire cohort were performed by conditional logistic regression, adjusted for BMI and smoking status. Subset analyses, stratified by follow-up time, were performed by non-conditional logistic regression to maintain statistical power. Non-conditional analyses were adjusted for matching factors, BMI, smoking status, and analysis plate. Tests for linear trend were calculated using immune marker quartiles as a continuous variable.

References

1. Vermeulen R, Saberi Hosnijeh F, Bodinier B, et al. Pre-diagnostic blood immune markers, incidence and progression of B-cell lymphoma and multiple myeloma: Univariate and functionally informed multivariate analyses. Int J Cancer. 2018;143(6):1335-1347.
2. Lubin JH, Colt JS, Camann D, et al. Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. Environmental Health Perspectives. 2004;112(17):1691-1696.
3. Sterne JAC, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009;338.

Supplementary Table S1. Selected disease and patient characteristics at diagnosis

	Myeloma cases	
Characteristics at diagnosis		
N (%)	65	(100)
Mean age at diagnosis, years (range)	63.3	(42-84)
Year of diagnosis, n (%)		
1997 - 2002	12	(18.4)
2003 - 2008	28	(43.1)
2009 - 2013	25	(38.5)
International staging system score, n (%)		
I	35	(53.8)
II	13	(20.0)
III	15	(23.1)
Serum M-protein, n (%)		
IgG	41	(63.1)
IgA	11	(16.9)
IgD	1	(1.5)
IgM	1	(1.5)
Normal or subclass unknown	11	(17.0)
Mean serum M-protein concentration, g/L (SD)	30.8	(19.2)
Immunoglobulin light chain, n (%)		
Kappa	36	(64.3)
Lambda	20	(35.7)
Detectable M-protein in urine, n (%)	44	(67.7)
Serum free light chain ratio, n (%)		
Abnormal	14	(21.5)
Normal	1	(1.6)
Not performed	50	(76.9)
Mean clonal bone marrow plasma cells, % (SD)	31.4	(23.0)
CRAB features, n (%)		
0	22	(33.8)
1	19	(29.2)
≥2	24	(37.0)
Treatment and follow-up characteristics		
Number of treatment lines received, mean (range)	3.5	(0-9)
Patients that received ASCT, n (%)	34	(52.3)
Patients that received proteasome inhibitors, n (%)	43	(66.2)
Patients that received immunomodulatory drugs, n (%)	39	(60.0)
Disease status of patients deceased and alive by August 2017, n/n (%/%)	39/26	(60.0/40.0)
Complete response	0/4	(0/15.4)
Very good partial response	0/3	(0/11.5)
Partial response	0/4	(0/15.4)
Stable disease	3/5	(7.7/19.2)
Progressive disease	35/4	(89.7/15.4)
Smoldering multiple myeloma	1/6	(2.6/23.1)

CRAB, elevated Calcium, Renal failure, Anaemia, Bone lesions; ASCT, autologous stem cell transplant;

Supplementary Table S2. Cut-off values (bold) for repeated marker measures (progressing vs. non-progressing to MM)

MCP-3	Sens	1-Spec	**VEGF**	Sens	1-Spec	**FGF-2**	Sens	1-Spec	**Fractalkine**	Sens	1-Spec	**TGF-α**	Sens	1-Spec
2,4400	1,000	1,000	42,9500	1,000	1,000	20,3400	1,000	1,000	61,6181	1,000	1,000	-,9094	1,000	1,000
5,0000	1,000	,983	50,4700	1,000	,983	36,0550	1,000	,983	65,2991	1,000	,983	,1103	1,000	,983
7,1123	1,000	,966	77,5966	1,000	,966	64,7550	1,000	,966	92,8900	1,000	,966	,1600	1,000	,966
9,7423	1,000	,948	98,4266	1,000	,948	86,4600	1,000	,948	123,2150	1,000	,948	,2100	1,000	,948
14,9750	1,000	,931	100,4750	1,000	,931	96,6600	1,000	,931	130,9900	1,000	,931	,2401	1,000	,931
18,2263	1,000	,914	104,5500	1,000	,914	102,9450	1,000	,914	135,8550	1,000	,914	,2551	1,000	,914
18,8613	1,000	,897	115,4650	1,000	,897	106,9500	1,000	,897	141,7650	1,000	,897	,2613	1,000	,897
19,5900	1,000	,879	142,7150	1,000	,879	107,2250	1,000	,879	145,2400	1,000	,879	,3232	1,000	,879
19,8800	1,000	,862	162,6000	1,000	,862	107,7900	1,000	,862	151,0600	1,000	,862	,4360	1,000	,862
20,6590	1,000	,845	163,9650	1,000	,845	111,4100	1,000	,845	160,9000	1,000	,845	,4971	1,000	,845
21,3990	1,000	,828	166,2500	1,000	,828	117,7600	1,000	,828	166,1100	1,000	,828	,5780	1,000	,828
21,9100	1,000	,810	169,1850	1,000	,810	126,5950	1,000	,810	179,0450	1,000	,810	,6526	1,000	,810
22,3750	1,000	,793	183,8300	1,000	,793	132,7050	1,000	,793	191,8750	1,000	,793	,6726	1,000	,793
23,0850	1,000	,776	197,9900	1,000	,776	134,8050	1,000	,776	194,5700	1,000	,776	,7750	1,000	,776
23,9550	1,000	,759	204,0900	1,000	,759	136,6100	1,000	,759	198,3250	1,000	,759	,8700	1,000	,759
24,9650	1,000	,741	212,2850	1,000	,741	138,1850	1,000	,741	201,3750	1,000	,741	,9050	1,000	,741
27,0950	1,000	,724	217,0100	1,000	,724	140,2700	1,000	,724	203,9050	1,000	,724	,9450	1,000	,724
28,5650	1,000	,707	219,6450	1,000	,707	143,2600	1,000	,707	208,2200	1,000	,707	,9850	1,000	,707
28,8300	1,000	,690	228,8950	1,000	,690	145,6300	1,000	,690	212,7200	1,000	,690	1,0700	1,000	,690
29,5800	1,000	,672	247,8450	1,000	,672	147,6450	1,000	,672	216,8400	1,000	,672	1,1500	1,000	,672
30,2900	1,000	,655	261,5150	,857	,672	152,3500	1,000	,655	222,6000	1,000	,655	1,1705	1,000	,655
30,6900	1,000	,638	266,3850	,857	,655	159,6400	1,000	,638	228,1600	1,000	,638	1,2105	1,000	,638
32,3550	1,000	,621	268,0400	,857	,638	165,1950	1,000	,621	231,5100	1,000	,621	1,2950	1,000	,621
33,9350	1,000	,603	271,2400	,857	,621	167,8450	1,000	,603	236,0200	1,000	,603	1,3600	,857	,621
34,7200	1,000	,586	275,9600	,857	,603	169,7150	,857	,603	248,4650	1,000	,569	1,4550	,857	,603
35,3800	1,000	,569	281,3300	,857	,586	173,4450	,857	,586	258,2300	1,000	,552	1,6010	,857	,586
37,9400	1,000	,552	285,2550	,857	,569	177,5300	,857	,569	259,5350	,857	,552	1,6910	,857	,569
40,5500	1,000	,534	286,0000	,857	,552	178,6950	,857	,552	262,6600	,857	,534	1,7596	,857	,552
41,3700	1,000	,517	289,1450	,857	,534	180,0550	,857	,534	269,1000	,857	,517	1,8304	,857	,534
42,1200	1,000	,500	294,0050	,857	,517	184,8500	,857	,517	273,6450	,857	,500	1,8758	,857	,517
42,2950	1,000	,483	**296,3800**	,857	,500	188,9750	,857	,500	277,2650	,857	,483	1,9400	,857	,500
43,4150	1,000	,466	298,6850	,714	,500	192,2500	,857	,483	283,6100	,857	,466	2,0050	,857	,483
44,7850	1,000	,431	302,5300	,714	,483	199,3800	,857	,466	287,7500	,857	,448	2,0450	,857	,466
45,4750	,857	,431	304,8700	,571	,483	204,3350	,857	,448	289,0950	,857	,431	2,1400	,857	,448
46,8400	,857	,414	306,1350	,571	,466	205,7550	,714	,448	289,6900	,857	,414	2,3250	,857	,431
47,9250	,857	,397	307,5900	,571	,448	207,6450	,714	,431	**291,6400**	,857	,397	2,5250	,857	,414
48,5450	,714	,397	314,3450	,571	,431	210,8250	,714	,414	298,7950	,714	,397	2,6650	,857	,397
49,1200	,571	,397	323,7800	,571	,414	221,7700	,714	,397	306,2400	,714	,379	3,0300	,857	,379
49,2750	,571	,379	328,8650	,571	,397	**233,6750**	,714	,379	309,8750	,571	,362	3,3550	,857	,362
50,1300	,571	,362	332,7600	,571	,379	237,4500	,714	,362	312,0800	,571	,345	3,4050	,857	,345
50,9450	,429	,362	338,6900	,571	,362	238,5500	,571	,362	316,7850	,571	,328	**3,5300**	,857	,328
51,7050	,429	,345	347,1900	,429	,362	239,4900	,571	,345	322,2600	,571	,310	3,7200	,714	,328
52,9050	,286	,345	354,9550	,429	,345	240,8250	,571	,328	328,8850	,571	,293	3,8550	,714	,310
53,7300	,286	,328	358,6250	,429	,328	243,1600	,571	,310	339,2350	,571	,276	3,9050	,714	,293
54,8000	,286	,310	360,1350	,429	,310	246,6250	,429	,310	345,9100	,571	,259	3,9450	,571	,276
55,6850	,286	,293	368,3050	,429	,293	248,3050	,429	,293	348,4300	,429	,259	3,9800	,571	,259
57,0100	,286	,276	375,4250	,429	,276	248,6100	,429	,276	352,4750	,429	,241	4,1450	,571	,241
58,2850	,286	,259	376,7900	,429	,259	256,5450	,429	,259	358,1850	,429	,224	4,3550	,571	,224
58,5150	,286	,241	397,4950	,429	,241	265,1500	,429	,241	361,4150	,286	,224	4,4400	,571	,207
60,3500	,143	,241	425,2250	,429	,224	276,6700	,429	,224	363,4400	,286	,207	4,4650	,571	,190
62,1950	,143	,224	437,0850	,429	,207	289,4350	,429	,207	375,0750	,143	,207	4,6650	,429	,190
64,0750	,143	,207	440,9900	,429	,190	294,0600	,429	,190	388,1750	,143	,190	4,9500	,429	,172
65,9850	,000	,207	441,5450	,429	,172	303,9350	,429	,172	391,9800	,143	,172	5,0550	,429	,155
67,5050	,000	,190	441,9750	,286	,172	311,4950	,429	,155	397,3750	,143	,155	5,1700	,429	,138
69,4650	,000	,172	443,3000	,286	,155	321,0450	,429	,138	404,2150	,143	,138	5,2850	,286	,138
70,6950	,000	,155	444,9950	,286	,138	331,3200	,286	,138	410,1550	,000	,138	5,4150	,286	,121
75,5200	,000	,138	462,9800	,143	,138	335,0100	,286	,121	431,2900	,000	,121	5,6200	,286	,103
79,9250	,000	,121	498,7900	,143	,121	343,5600	,286	,103	458,3150	,000	,103	5,8550	,143	,103
80,6000	,000	,103	535,6050	,143	,103	351,3550	,286	,086	469,8800	,000	,086	6,1850	,143	,086
85,7000	,000	,086	559,0350	,143	,086	361,2150	,286	,069	476,4400	,000	,069	6,6650	,000	,086
93,0450	,000	,069	578,7400	,143	,069	381,9200	,143	,069	502,0450	,000	,052	7,5300	,000	,069
101,2150	,000	,052	608,4000	,143	,052	397,2200	,143	,052	569,2800	,000	,034	8,7050	,000	,052
117,1100	,000	,034	630,2900	,143	,034	405,7350	,143	,034	626,6700	,000	,017	9,5650	,000	,034
135,1550	,000	,017	637,2200	,000	,034	428,7300	,000	,034	638,8600	,000	,000	12,1100	,000	,017
143,7500	,000	,000	666,6700	,000	,017	450,9050	,000	,017				15,4000	,000	,000
			697,0200	,000	,000	457,1500	,000	,000						

Supplementary Table S3. Myeloma risk by immune marker quartiles at baseline and repeated blood sample collection

Analytes	Baseline[a]	All cases		12 - 24[c]		Repeated[a]	All cases		0 - 4[c]	
(pg/mL)	$N_{Controls}$	N	OR[b] (95% CI)	N	OR[d] (95% CI)	$N_{Controls}$	N	OR[b] (95% CI)	N	OR[d] (95% CI)
MCP-3										
≤37.92	16	19	Ref	11	Ref	18	26	Ref	17	Ref
37.93-55.25	17	22	1.13 (0.40-3.17)	14	1.61 (0.53-4.93)	19	19	0.70 (0.29-1.72)	10	0.61 (0.21-1.76)
55.26-73.66	16	14	0.60 (0.21-1.74)	7	0.37 (0.10-1.41)	13	12	0.62 (0.23-1.70)	4	0.35 (0.09-1.36)
≥73.67	16	10	0.42 (0.12-1.46)	5	0.23 (0.05-1.02)	15	8	0.38 (0.13-1.09)	3	0.25 (0.06-1.06)
P_{trend}[e]			0.120		0.038			0.075		0.032
VEGF										
≤242.99	16	13	Ref	7	Ref	17	19	Ref	10	Ref
243.01-351.88	17	20	1.76 (0.63-4.96)	11	1.79 (0.52-6.19)	21	22	0.91 (0.37-2.23)	17	1.39 (0.49-3.93)
351.89-447.66	16	12	0.94 (0.33-2.72)	6	0.92 (0.24-3.54)	8	13	1.37 (0.46-4.06)	5	1.18 (0.29-4.83)
≥447.67	16	20	1.65 (0.57-4.78)	13	1.77 (0.50-6.19)	19	11	0.51 (0.18-1.41)	2	0.17 (0.03-0.93)
P_{trend}			0.591		0.611			0.293		0.053
FGF-2										
≤170.32	16	10	Ref	5	Ref	19	25	Ref	16	Ref
170.33-244.05	17	25	2.43 (0.87-6.80)	14	3.13 (0.83-11.82)	17	18	0.90 (0.37-2.19)	12	0.88 (0.31-2.52)
244.06-327.49	16	15	1.52 (0.49-4.69)	8	1.72 (0.43-6.95)	12	11	0.72 (0.22-2.39)	4	0.37 (0.10-1.46)
≥327.50	16	15	1.44 (0.44-4.69)	10	1.94 (0.48-7.91)	17	11	0.50 (0.20-1.31)	2	0.16 (0.03-0.82)
P_{trend}			0.756		0.711			0.158		0.012
Fractalkine										
≤251.50	16	16	Ref	10	Ref	26	25	Ref	16	Ref
251.51-314.59	17	21	1.09 (0.41-2.90)	11	1.14 (0.36-3.60)	12	17	1.48 (0.57-3.85)	7	1.06 (0.33-3.38)
314.60-445.94	16	20	1.32 (0.47-3.70)	12	1.63 (0.50-5.31)	15	17	1.24 (0.53-2.93)	9	0.80 (0.27-2.36)
≥445.95	16	8	0.30 (0.07-1.26)	4	0.31 (0.07-1.35)	12	6	0.52 (0.17-1.61)	2	0.28 (0.05-1.48)
P_{trend}			0.291		0.321			0.491		0.183
TGF-α										
≤1.66	16	14	Ref	7	Ref	17	25	Ref	15	Ref
1.67-3.13	16	19	1.31 (0.47-3.65)	11	1.54 (0.45-5.28)	18	12	0.43 (0.16-1.17)	10	0.66 (0.22-1.97)
3.14-5.30	17	20	1.30 (0.43-4.00)	14	1.86 (0.57-6.11)	16	19	0.82 (0.30-2.27)	7	0.47 (0.15-1.51)
≥5.31	16	12	0.87 (0.24-3.10)	5	0.58 (0.14-2.47)	14	9	0.38 (0.12-1.18)	2	0.17 (0.03-0.95)
P_{trend}			0.828		0.648			0.226		0.030

[a]Baseline and repeated samples categorized into quartiles based on the distribution among controls at baseline.

[b]Odds ratios were calculated by conditional logistic regression adjusted for body mass index and smoking status.

[c]Analyses restricted to myeloma cases with sample collection long and short before diagnosis.

[d]Odds ratios were calculated by non-conditional logistic regression adjusted for age, sex, sample collection date, analysis plate, body mass index, and smoking status.

[e]All P_{trend} values were calculated by including immune marker quartiles as a continuous variable.

Supplementary Table S4. Spearman correlation coefficients among all markers including all samples (N=260)

	MCP-3	MIP-1α	MIP-1β	IL-13	IL-10	TNF-α	FGF-2	VEGF	TGF-α	Fractalkine
MCP-3	1,000									
MIP-1α	,649	1,000								
MIP-1β	,604	,764	1,000							
IL-13	,755	,690	,523	1,000						
IL-10	,732	,750	,688	,731	1,000					
TNF-α	,576	,773	,837	,514	,753	1,000				
FGF-2	,722	,697	,792	,503	,616	,681	1,000			
VEGF	,611	,679	,729	,528	,580	,611	,825	1,000		
TGF-α	,705	,812	,751	,677	,769	,828	,732	,685	1,000	
Fractalkine	,602	,735	,661	,675	,628	,689	,647	,668	,794	1,000

Supplementary Figure S1. Receiver operating curves (ROC) for prediagnostic repeated measures of immune markers between 58 individuals who progressed to MM and 7 individuals who did not progress until latest clinical follow-up