Markers of T cell and monocyte/macrophage activation are associated with adverse outcome, but give limited prognostic value in anemic patients with heart failure - results from the RED-HF study

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Supplementary Table 1. Characteristics of the enzyme immunoassays showing levels (as %) vs. comparison levels.

	Non-fasting, % of fasting	Noon, % of morning	4h bench, % of "fresh"	24h bench, % of "fresh"	4h fridge, % of "fresh"	24h fridge, % of "fresh"	3 freeze-thaw cycles, % of fresh
sCD163	97%	120%*	91%	85%	82%	80%*	99%
MIF	98%	102%	103%	113%	99%	90%	105%
Granulysin	95%	123%*	100%	102%	99%	91%	87%
sIL2R	98%	100%	113*	102%	92%	89%	92%
ALCAM	90%	134%*	92%	83%	84%	82%*	97%

The effect of post-prandial and diurnal variation and time exposure were assessed in 6 heathy individuals while the effect of freeze thaw cycles was assessed in 4 heathy individuals. *$p<0.05$.

Supplementary Table 2. Characteristics of the patients (n=1582) at baseline according to tertiles of sCD163, MIF, Granulysin, sIL2R, and ALCAM in plasma.

Characteristic		Tertile 1	Tertile 2	Tertile 3	P-value
Tertile limits	sCD163**	<0.49	0.49-0.86	>0.86	
	MIF*	<1.30	1.30-2.13	>2.13	
	Granulysin*	<1.20	1.20-1.77	>1.77	
	sIL2R*	<218	218-357	>357	
	ALCAM*	<150	150-202	>202	
Age yrs, mean±SD	sCD163	70.0±12.2	69.5±11.2	68.9±11.5	0.061
	MIF	70.1±11.3	69.2±12.2	69.0±11.4	0.143
	Granulysin	69.0±11.6	68.8±11.8	70.6±11.4	0.019
	sIL2R	66.7±12.5	70.5±11.3	71.3±10.4	<0.001
	ALCAM	69.6±11.7	69.6±11.9	69.3±11.2	0.639
Male sex, %	sCD163	44	44	42	0.434
	MIF	44	43	43	0.980
	Granulysin	36	48	47	<0.001
	sIL2R	51	44	35	<0.001
	ALCAM	43	40	47	0.083
Race (white/black), %	sCD163	71/14	68/10	60/6	<0.001
	MIF	71/9	66/10	62/11	0.002
	Granulysin	70/7	65/10	64/13	0.004
	sIL2R	58/16	67/8	74/6	<0.001
	ALCAM	75/10	65/10	59/10	<0.001
BMI±SD, kg/m^2	sCD163	27.1±5.7	27.2±6.1	27.0±5.4	0.986
	MIF	27.0±5.4	27.0±5.7	27.3±6.1	0.992
	Granulysin	26.9±5.4	27.3±5.9	27.0±5.9	0.814
	sIL2R	27.1±5.8	27.0±5.7	27.1±5.7	0.867
	ALCAM	27.4±5.7	26.9±5.5	26.9±6.0	0.074
NYHA (III or IV), %	sCD163	69	66	64	0.085
	MIF	68	64	67	0.411
	Granulysin	68	65	67	0.579
	sIL2R	65	65	69	0.213
	ALCAM	71	64	64	0.020
LVEF, %	sCD163	30.3±6.9	29.9±6.9	30.6±6.8	0.485
	MIF	30.0±6.7	30.5±6.9	30.3±7.0	0.384
	Granulysin	30.2±6.7	30.9±6.8	29.8±6.9	0.024
	sIL2R	30.3±6.8	30.1±6.8	30.4±6.9	0.738
	ALCAM	30.3±6.9	30.4±6.7	30.2±6.9	0.913
Ischemic HF, %	sCD163	69	72	74	0.079
	MIF	72	72	70	0.695
	Granulysin	71	71	73	0.459
	sIL2R	64	75	76	<0.001
	ALCAM	68	76	71	0.022
	sCD163	75	74	73	0.316
	MIF	74	73	76	0.451

Hypertension, %	Granulysin	72	74	76	0.104
	sIL2R	73	73	76	0.297
	ALCAM	78	73	71	0.013
Diabetes, %	sCD163	41	46	47	0.032
	MIF	44	42	48	0.147
	Granulysin	48	44	42	0.04
	sIL2R	40	46	48	0.006
	ALCAM	43	43	48	0.184
Atrial fibrillation or Flutter, %	sCD163	34	30	32	0.33
	MIF	33	29	33	0.302
	Granulysin	31	27	37	0.002
	sIL2R	26	32	37	<0.001
	ALCAM	32	31	32	0.899
Stroke, %	sCD163	8	8	7	0.568
	MIF	8	8	7	0.301
	Granulysin	8	7	8	0.983
	sIL2R	5	9	10	0.004
	ALCAM	8	6	8	0.885
MI last 6 mo., %	sCD163	33	39	38	0.118
	MIF	33	36	40	0.053
	Granulysin	35	35	40	0.152
	sIL2R	32	33	45	<0.001
	ALCAM	32	40	38	0.026
Cancer, %	sCD163	7	7	7	0.712
	MIF	7	6	8	0.468
	Granulysin	8	6	7	0.696
	sIL2R	4	8	9	0.002
	ALCAM	6	6	9	0.146
ACE inhib. or ARB, %	sCD163	92	90	89	0.061
	MIF	93	90	87	0.001
	Granulysin	91	91	89	0.298
	sIL2R	94	92	84	<0.001
	ALCAM	93	89	89	0.019
Beta-blocker, %	sCD163	84	86	86	0.612
	MIF	85	85	85	0.739
	Granulysin	85	85	85	0.839
	sIL2R	85	86	84	0.429
	ALCAM	87	85	83	0.063
Diuretic, %	sCD163	90	92	93	0.187
	MIF	90	91	94	0.038
	Granulysin	89	92	93	0.036
	sIL2R	89	93	93	0.014
	ALCAM	91	92	92	0.295
Systolic BP (mmHg), mean±SD	sCD163	121.4±17.5	120.1±18.8	118.6±18.0	0.01
	MIF	120.8±18.0	119.7±18.0	119.7±18.4	0.463
	Granulysin	120.8±18.7	121.1±17.9	118.2±17.6	0.010

	sIL2R	120.2±17.6	119.7±18.0	120.3±18.8	0.931
	ALCAM	120.5±17.7	120.6±18.2	119.1±18.5	0.170
Diastolic BP (mmHg), mean±SD	sCD163	69.3±11.5	69.8±11.1	69.2±10.5	0.694
	MIF	69.9±10.9	68.8±11.4	69.5±10.7	0.515
	Granulysin	69.8±11.4	69.4±10.5	69.0±11.0	0.27
	sIL2R	70.7±10.8	68.8±11.2	68.7±10.9	0.004
	ALCAM	69.3±11.2	70.0±11.0	68.9±10.8	0.442
Heart rate (b.p.m.), mean±SD	sCD163	71.3±11.5	72.7±11.2	72.3±10.9	0.099
	MIF	71.0±10.7	71.4±11.0	73.8±11.8	<0.001
	Granulysin	72.7±11.1	72.1±11.0	71.6±11.5	0.208
	sIL2R	72.3±11.0	71.3±11.1	72.8±11.5	0.174
	ALCAM	71.3±11.6	72.7±10.9	72.2±11.1	0.074
Biochemistry					
Creatinine (mg/dL), mean±SD	sCD163	1.4±0.5	1.5±0.5	1.5±0.6	0.002
	MIF	1.4±0.5	1.5±0.6	1.5±0.6	<0.001
	Granulysin	1.4±0.5	1.5±0.6	1.5±0.6	<0.001
	sIL2R	1.2±0.4	1.5±0.5	1.7±0.6	<0.001
	ALCAM	1.4±0.5	1.5±0.6	1.6±0.6	<0.001
eGFR (mL/min/1.73m^2), mean±SD	sCD163	52.8±21.7	48.7±19.8	49.3±23.0	0.001
	MIF	53.7±20.8	49.3±22.6	48.0±21.1	<0.001
	Granulysin	53.2±21.9	50.6±22.0	47.3±20.7	<0.001
	sIL2R	60.4±22.6	49.3±19.9	41.2±17.7	<0.001
	ALCAM	53.9±21.6	50.5±21.1	46.4±21.7	<0.001
Hemoglobin (g/dL), mean±SD	sCD163	11.1±0.7	11.0±0.7	11.0±0.7	0.267
	MIF	11.1±0.7	11.0±0.7	11.0±0.7	0.305
	Granulysin	11.0±0.7	11.1±0.7	11.0±0.7	0.151
	sIL2R	11.2±0.7	11.0±0.7	10.9±0.8	<0.001
	ALCAM	11.1±0.7	11.1±0.7	11.0±0.7	0.006
Transferrin saturation (%),mean±SD	sCD163	27.7±10.5	26.6±10.7	26.6±11.2	0.009
	MIF	27.6±10.9	26.6±10.3	26.8±11.5	0.058
	Granulysin	27.1±11.9	27.5±10.8	26.4±9.9	0.97
	sIL2R	27.6±10.5	27.1±11.5	26.3±10.7	0.008
	ALCAM	27.1±10.5	27.4±11.3	26.4±10.7	0.082
Platelets (10^9/L), mean±SD	sCD163	236.9±78.1	238.4±79.2	218.7±79.0	<0.001
	MIF	228.2±78.5	231.4±81.0	234.7±78.3	0.05
	Granulysin	230.4±74.6	230.4±79.6	233.7±83.5	0.853
	sIL2R	239.6±80.3	232.4±77.7	222.4±78.9	<0.001
	ALCAM	235.2±74.7	233.3±80.1	225.6±82.6	0.008
WBC (10^9/L), mean±SD	sCD163	6.7±2.2	6.8±2.2	6.7±2.1	0.897
	MIF	6.4±2.0	6.8±2.2	7.0±2.3	<0.001
	Granulysin	6.6±2.0	6.7±2.2	6.8±2.3	0.275
	sIL2R	6.5±2.0	6.9±2.3	6.8±2.3	0.011
	ALCAM	6.6±2.2	7.0±2.4	6.5±1.9	0.833
hsCRP (mg/L), median (IQR)	sCD163	2.3(0.9, 6.1)	2.7(1.1, 6.7)	3.1(1.2, 7.8)	0.002
	MIF	2.0 (0.8, 4.8)	2.9 (1.0, 7.4)	3.5 (1.4, 9.4)	<0.001
	Granulysin	2.4 (0.9, 6.2)	2.5 (1.0, 6.6)	3.1 (1.3, 7.6)	0.011

	sIL2R	2.1(0.8, 5.0)	2.4(1.0, 5.8)	3.8(1.5, 12.1)	<0.001
	ALCAM	2.6(1.0, 6.4)	2.6(0.9, 6.8)	2.9(1.2, 7.3)	0.249
NT-proBNP (pg/mL), median (IQR)	sCD163	1611(551, 3765)	1976(667, 4242)	1995(777, 4920)	0.006
	MIF	1577 (518, 3405)	1891 (696, 4570)	2184 (753, 5482)	<0.001
	Granulysin	1815 (569, 4374)	1633 (569, 3777)	1995(823, 4530)	0.005
	sIL2R	1071(265, 2677)	1827(727, 4017)	2823(1181, 6803)	<0.001
	ALCAM	1520(486, 3644)	1743(661, 3767)	2283(809, 5703)	<0.001
hsTnT (ng/ml), median (IQR)	sCD163	24.1(14.7, 39)	24.5(14.6, 42.4)	28.2(16.8, 46.9)	0.005
	MIF	22.5(13.4, 38.1)	27.5(14.9, 44.2)	27.4(17.5, 46.5)	<0.001
	Granulysin	24.7(14.6, 41.2)	24.6(14.0, 41.7)	27.0(17.0, 45.1)	0.024
	sIL2R	18.2(10.8, 29.4)	26(16.2, 40.0)	36.9(22.5, 54.3)	<0.001
	ALCAM	22.5(13.4, 38.1)	25.7(15.2, 42.3)	29.5(17.3, 50.3)	<0.001

Values are given as mean ± SD for continuous variables and % of cases for categorical variables. ACE, angiotensin-converting enzyme; ALCAM, activated leukocyte cell adhesion molecule; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitive troponin T; LVEF, left ventricular ejection fraction; MIF, macrophage migration inhibitory factor; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; sCD163, soluble cluster of differentiation 163; sIL2R, soluble IL-2 receptor; WBC, white blood cell; WHF, worsening heart failure.
* ng/mL, ** μg/mL.

Supplementary Table 3. Predictors of leukocyte activation markers

| Characteristic	sCD163	MIF	Granulysin	sIL2R	ALCAM
Age yrs	0.002*	-0.02*			
Male sex	0.1*		-0.2**	-37*	-16.0***
Race (white)	0.14***		-0.3***	105***	-6.4***
LVEF				2.6*	
Atrial fibrillation or flutter			0.1**		
Heart rate		0.02***			
MI last 6 mo.			0.1*	29.2*	
Systolic BP			-0.002*		
Creatinine	0.18*			104***	17.5***
eGFR	0.004*		-0.01***		
Hemoglobin				-35**	
Platelets	0.001***				-0.1**
hsCRP, log	0.044**			28.3***	
NT-proBNP, log				29.2***	
hsTnT, log	0.046**	0.30*		34.2**	8.4***

ALCAM, activated leukocyte cell adhesion molecule; BP, blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitive troponin T; LVEF, left ventricular ejection fraction; MIF, macrophage migration inhibitory factor; NT-proBNP, N-terminal pro-brain natriuretic peptide; sCD163, soluble cluster of differentiation 163; sIL2R, soluble IL-2 receptor. *p<0.05, **p<0.01, ***p<0.001

Supplementary Table 4. Association between baseline levels of leukocyte markers (continuous) and outcomes in patients with (DM) and without diabetes (Non-DM). Hazard ratios (HRs) and 95% confidence interval (CI) per log change are shown as the final fully-adjusted model including clinical variables, hsCRP, hsTnT and NT-proBNP as described in statistical methods.

HR(95% CI)		Primary composite	Secondary composite	All-cause death	Cardiovascular death
sil2r	DM	0.99 (0.88,1.12)	0.96 (0.85,1.08)	1.05 (0.92,1.20)	1.01 (0.87,1.17)
	Non-DM	1.03 (0.93,1.14)	1.03 (0.92,1.15)	0.95 (0.84,1.06)	0.93 (0.82,1.05)
Alcam	DM	1.08 (0.94,1.24)	1.05 (0.90,1.22)	1.00 (0.86,1.18)	0.97 (0.81,1.15)
	Non-DM	1.00 (0.89,1.14)	1.00 (0.87,1.14)	1.03 (0.90,1.18)	1.04 (0.90,1.21)
sCD163	DM	0.93 (0.80,1.07)	0.91 (0.78,1.07)	0.99 (0.84,1.17)	0.94 (0.80,1.13)
	Non-DM	1.11 (0.99,1.24)	1.11 (0.98,1.24)	1.13 (1.00,1.28)*	1.13 (0.99,1.29)
Granulysin	DM	0.95 (0.85,1.05)	0.94 (0.84,1.05)	1.00 (0.88,1.14)	1.00 (0.87,1.15)
	Non-DM	0.88 (0.80,0.97)	0.87 (0.78,0.97)	0.90 (0.81,1.00)	0.87 (0.78,0.98)
MIF	DM	1.02 (0.91,1.13)	1.02 (0.91,1.14)	1.03 (0.92,1.17)	1.06 (0.93,1.20)
	Non-DM	1.09 (0.98,1.21)	1.05 (0.95,1.18)	1.08 (0.96,1.21)	1.05 (0.93,1.19)

ALCAM, activated leukocyte cell adhesion molecule; MIF, macrophage migration inhibitory factor; sCD163, soluble cluster of differentiation 163; sIL2R, soluble IL-2 receptor.*p<0.05

Supplemental Figure 1. Marker levels (ng/mL) according to year of isolationand distribution within each year (blue) and according to time of day isolation was performed (red). *p<0.05 vs. 0600-0900.
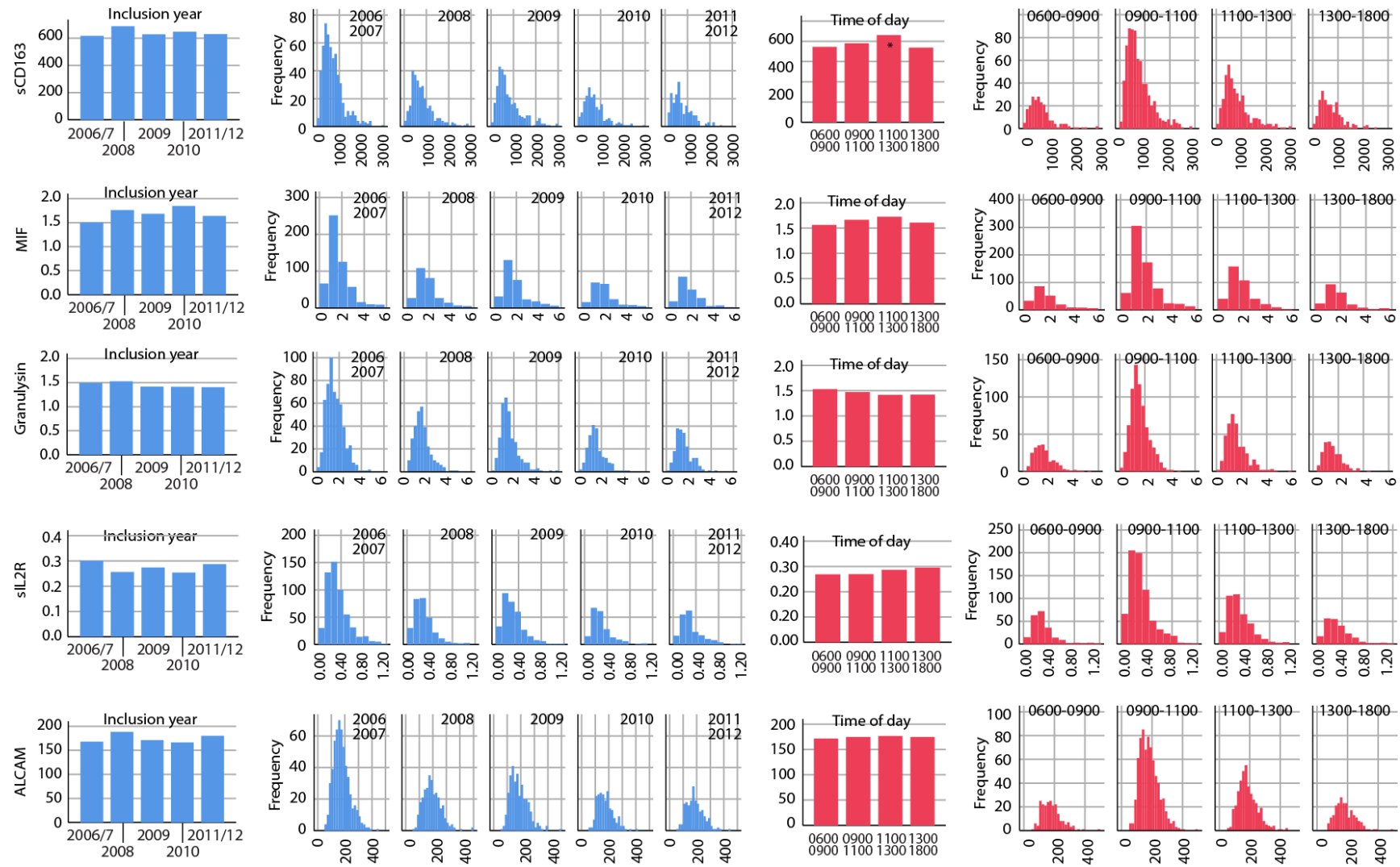
Supplementary Figure 1. Restricted cubic spline analysis of baseline leukocyte markers showing tertile (T1, T2 and T3) limits.