T cell and monocyte/macrophage activation markers associate with adverse outcome, but give limited prognostic value in anemic patients with heart failure - results from RED-HF

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Running head: monocyte/macrophage markers in HF

Abstract

Background: Activated leukocytes may contribute to the development and progression of heart failure (HF). We investigated the predictive value of circulating levels of stable and readily detectable markers reflecting both monocyte/macrophage and T-cell activity, on clinical outcomes in heart failure (HF) patients with reduced ejection fraction (HFrEF). **Methods:** The association between baseline plasma levels of soluble CD163 (sCD163), macrophage migration inhibitory factor (MIF), granulysin, soluble interleukin-2 receptor (sIL-2R), and activated leukocyte cell adhesion molecule (ALCAM) and the primary endpoint of death from any cause or first hospitalization for worsening of HF was evaluated using multivariable Cox proportional hazard models in 1541 patients with systolic HF and mild to moderate anemia, enrolled in the Reduction of Events by darbepoetin alfa in Heart Failure (RED-HF) trial. Modifying effects and interaction with darbepoetin alfa treatment were also assessed. **Results:** All leukocyte markers, except granulysin, were associated with the primary outcome and all-cause death in univariate analysis (all p<0.01) and remained significantly associated in multivariable analysis adjusting for conventional clinical variables (e.g. age, gender, BMI, NYHA class, creatinine, LVEF, etiology) and CRP. However, after final adjustment for TnT and NT-proBNP no associations were found with outcomes. No interaction with darbepoetin alpha treatment was observed for any marker.

Conclusions: Leukocyte activation markers sCD163, MIF, sIL-2R, and ALCAM were associated with adverse outcome in patients with HFrEF, but add little as prognostic markers on top of established biochemical risk markers.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT00358215

Keywords: leukocyte, monocyte, macrophage, t cell, heart failure, prognosis

Heart failure (HF) is a progressive disorder with an element of immune activation and a persistent low grade inflammation [1-4]. Activated monocytes/macrophages and T cells release a variety of inflammatory cytokines that have direct effects on cardiac cells, promoting hypertrophy, fibrosis, and apoptosis [5,6,3]. Cytokine secretion from monocytes/macrophages, T cells as well as other leukocyte subpopulations and myocardial cells initiate further leukocyte mobilization and recruitment to the myocardium, which creates a vicious circle, further promoting the development of HF [3].

Multiple circulating cytokines are increased in HF patients systemically but they frequently circulate at relatively low levels which limits their analytical performance and discriminatory properties beyond established clinical markers of HF [7]. Some markers reflecting general or more specific leukocyte activation, circulate at readily detectable levels, but have been scarcely evaluated in large HF populations, with a few exceptions. Circulating myeloperoxidase, an enzyme primarily secreted by activated granulocytes, gave no prognostic information in adjusted analysis in 1513 HF patients with reduced ejection fraction (HFrEF) [8]. In contrast, galectin 3, which may partly reflect macrophage activation, has received wide attention in predicting adverse outcomes and response to therapy in HF patients potentially reflecting its ability to reflect both extracellular matrix (ECM) and inflammation [9].

Anemia is frequently observed in HF and associated with poor prognosis [10-12]. Based on the established role of persistent leukocyte activation in anemia [13] and its emerging role in the progression of HF, we hypothesized that plasma levels of stable and readily detectable markers of leukocyte activation, reflecting both monocyte/macrophage (soluble [s] CD163 [14], macrophage migration inhibitory factor [MIF] [15]) and/or T-cell (soluble interleukin 2 receptor [sIL-2R] [16], activated leukocyte cell adhesion molecule [ALCAM] [17], granulysin [18]) activity could represent clinically relevant inflammatory biomarkers in HF patients. We therefore evaluated if they could predict clinical outcomes in 1588 anemic patients with HFrEF in the Reduction of Events by darbepoetin alfa in Heart Failure (RED-HF) trial [19]. In general, the intervention with darbepoetin alfa did not show any effects on the primary end-point, but a secondary aim in this study was to evaluate if these markers could identify subgroups of patients who may still benefit from darbepoetin alfa treatment.

Materials and Methods

Patients and Study Procedures

The study design and baseline characteristics of the RED-HF trial have been reported in detail previously [20,21]. Patients were eligible for the study if they had the New York Heart Association (NYHA) functional class II-IV; systolic HF with left ventricular ejection fraction (LVEF) ≤ 40%; a hemoglobin level of 9.0 to 12.0 g per deciliter and were receiving guideline-recommended HF therapy. Major exclusion criteria were a transferrin saturation of less than 15%, evidence of bleeding or other correctable causes of anemia, a serum creatinine level of more than 3 mg per deciliter (265 µmol per liter), and a blood pressure of more than 160/100 mm Hg. Patients were randomly assigned in a 1:1 ratio to receive either darbepoetin alfa or placebo. The study drug was administered subcutaneously, with doses adjusted according to hemoglobin level, which was measured in a blinded fashion. Patients in the darbepoetin alfa group received a starting dose of 0.75 µg per kilogram of body weight once every 2 weeks until a hemoglobin level of 13.0 g per deciliter was reached on two consecutive visits. Thereafter, patients received monthly injections, according to an algorithm [20] that was designed to maintain a hemoglobin level of 13.0 g per deciliter (but not exceeding 14.5 g per deciliter). Patients in the placebo group received dose adjustments to mimic those in the darbepoetin alfa group. The trial complied with the Declaration of Helsinki and was approved by the Ethics Committees of the participating hospitals. All patients provided a written informed consent.

Study outcomes and definitions

The primary predefined outcome was a composite of death from any cause or first hospitalization for worsening of HF. The prespecified adjudicated secondary outcomes were 1) composite of death from cardiovascular (CV) causes or first hospitalization for worsening of HF, 2) death from any cause, 3) CV death. Details on the definition and adjudication of all outcomes have been described previously [19].

Blood sampling and biochemical analysis

All blood samples were non-fasting and all reported measurements, except for markers of leukocyte activation, were performed at a central laboratory, including N-terminal pro-brain natriuretic peptide (NT-proBNP) and high sensitivity assays for C-reactive protein (hsCRP) and troponin T (hsTnT) (Medical

Research Laboratories, Zaventem, Belgium). Blood samples for the measurement of the cytokines were collected in pyrogen-free EDTA tubes; centrifuged and isolated plasma was stored at –80°C until analyses. All samples were thawed an equal number of times (<3 times) and were on the bench <2 hours when preparing analysis. Circulating sCD163, MIF, granulysin, sIL-2R, and ALCAM were analyzed by enzyme immunoassays from R&D Systems (Minneapolis, MN, USA) with intra- and inter-assay coefficients of variation < 10%. The effect of postprandial and diurnal variation, time-delay on bench and in fridge and freeze-thaw cycles are presented in Supplemental Table 1 while marker levels according to inclusion year and time of day are presented in Supplemental Figure 1.

Statistical analysis

Trends across tertiles of the leukocyte markers were tested using Kruskal-Wallis H test, one-way ANOVA or chi-square depending on distribution and variable type (i.e. categorical or continuous). For comparing treatment effects on the markers, the Mann-Whitney U test was used on change values while Wilcoxon matched pairs test was used to assess longitudinal changes within groups. Stepwise linear regression was used to identify the most important predictors of the leukocyte markers. Kaplan-Meier curves were constructed to visualize and evaluate (log rank test) differences in survival. A restricted cubic spline analysis with three knots was undertaken on the primary outcome to assess linearity of risk. Survival analyses were performed using the Cox proportional hazard regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the leukocyte activation markers as a log-transformed continuous variables at baseline, which included mainly clinical variables at step one (age, gender, NYHA class, hospitalization for HF within 6 months, log serum creatinine, LVEF, etiology, body mass index (BMI), left bundle-branch block, history of atrial fibrillation or flutter, systolic blood pressure). At step two, log-transformed serum concentrations of hsCRP were included and finally, NT-proBNP and hsTnT were included at step three. A two-sided p-value <0.05 was considered to be significant. All statistical analyses were performed with the use of SAS software, version 9.2.

Results

Stability of T cell and monocyte/macrophage activation markers

As shown in Supplemental Figure 1, there was no association between time (i.e. year) or time of isolation during the day and marker level, except sCD163 was higher at midday. The distribution of marker level was similar at all time-points assessed. As shown in Supplemental Figure 1, no effect of fasting on biomarker levels was observed although there did appear to be some fluctuation over the day with increased levels of sCD163, Granulysin, ALCAM at noon (non-fasting). As also shown in Supplemental Table 1, time delay experiments, achieved by leaving samples on the bench or in the fridge for up to 24 hours, revealed no large major loss over time, although sCD163 and ALCAM tended to decline after 4 hours on bench and in the fridge and all markers was lower after 24 hours in the fridge. Finally, the effect of freeze thaw cycles had minor effects on marker levels.

Plasma levels of T cell and monocyte/macrophage activation markers in the RED-HF Cohort

Of the 2,278 patients enrolled in the RED-HF study, samples for measurement of the T cell and monocyte/macrophage activation markers were available for 1,582 (69%). The median plasma level of sCD163 at baseline in the overall population was 0.64 µg/mL (IQR 0.41 to 1.00 µg/mL); MIF 1.7 (1.1 to 2.5 ng/mL); granulysin 1.5 ng/mL (1.1 to 2.0 ng/mL); sIL-2R 277 pg/mL (189 to 409 pg/mL); ALCAM (175 ng/mL) 136 to 661 ng/mL. Clinical characteristics of the study population are given in Table 1 (Table 1). Clinical characteristics according to the tertiles of leukocyte markers are shown in Supplementary Table 2. Several demographic and clinical variables were associated with most of these markers, such as race, kidney function, hsCRP, hsTnT and NT-proBNP. None or few associations were found with NYHA class, LVEF and BMI. Of relevance to this patient population that also had anemia, high sIL-2R and ALCAM levels were associated with low hemoglobin levels, and high sIL-2R and sCD163 also correlated with low transferrin saturation.

Supplemental Table 3 shows predictors of levels of the monocyte/macrophage/T cell markers identified by stepwise linear regression. The four strongest significant predictors of the markers were, for sCD163: race, hsTnT, platelet count and hsCRP; for MIF: heart rate, hsTnT and age; for granulysin: eGFR, race, gender and previous MI; for sIL-2R: creatinine, NT-proBNP, hsCRP and race; for ALCAM:

creatinine, race, hsTnT and sex.

Association between baseline levels of T cell and monocyte/macrophage activation markers and outcome

in RED-HF trial

During a mean follow-up of 2.3 years (range 0.0-6.0 years), 798 patients reached the primary end point,

716 patients reached the composite of death from cardiovascular (CV) causes or first hospitalization for

worsening of HF, while 649 patients died and of these, 543 due to CV causes. Kaplan-Meier estimates for

the primary endpoint according to baseline tertiles of the measured leukocyte markers are presented in

Figure 1. A moderate association with the primary outcome was observed for all markers, with a more

defined tertile effect for sIL-2R, than for the other proteins. Also, for granulysin, the lowest outcome was

observed in tertile 2, suggesting a non-linear U-shaped association with outcome, which was confirmed

by a restricted cubic spline analysis (Supplementary Figure 2). For the other markers, the cubic spline

analysis against the primary endpoint revealed a linear increase in the first and second tertile, followed by

flattening of the curve around tertile 3 (Supplementary Figure 2).

When investigating the associations between log-transformed values of the inflammatory markers

and outcome, univariate Cox proportional hazard models including the markers separately showed that all

of them except granulysin were significantly associated with the primary outcome (Figure 2A), with the

strongest association for sIL-2R. These markers remained significantly associated with the primary

outcome and all-cause death in multivariable analysis, adjusting for a wide range of conventional clinical

variables (i.e. age, gender, BMI, NYHA class, HF hospitalization last 6 months, creatinine, LVEF, etiology,

left bundle-branch block, history of atrial fibrillation or flutter, systolic blood pressure (BP) and study drug)

(Step 1). sCD163, MIF, and sIL-2R were only marginally affected and still significantly associated with

both outcomes after further adjustment for hsCRP (Step 2). ALCAM remained significantly associated

with all-cause death, but not with the primary outcome. However, associations with outcomes were

markedly attenuated after the last adjustment for hsTnT and NT-proBNP showing no significant

associations with outcome (Step 3). Similar, but less prominent trends were seen for the secondary

endpoints of CV death or first hospitalization for worsening of HF and CV death, with the strongest

association for MIF and sIL-2R (Table 3).

As shown in Supplemental Table 2, there was a tendency for higher frequency of patients with high levels of leukocyte markers in patients with diabetes, significant for sCD163 and sIL-2R, while granulysin was lower. Based on the high frequency of diabetes in RED-HF we evaluated risk of adverse outcome in patients with (HbA1c mean±SD: 7.39±1.61 %) and without (5.75±0.64 %) diabetes separately, restricting analysis to fully adjusted models. As shown in Supplementary Table 4, only sCD163 displayed a trend of increased risk according to diabetes status with a higher risk in non-diabetic patients which was significant for all-cause death.

Effect of darbepoetin alfa on markers of T cell and monocyte/macrophage activation

Table 3 shows levels of the leukocyte markers at baseline and after 6 months treatment with darbepoetin alfa or placebo. There were no significant differences in the levels of the measured inflammatory markers between placebo and darbepoetin alfa groups at baseline. MIF increased after treatment with darbepoetin alfa giving significantly higher levels at 6 moths and a significant difference in change between the treatment groups, while sIL-2R decreased. Leukocyte markers did not change in placebo group. No association between change in any of the markers (i.e. decrease: <-15%, no change: 15%-15%, increase: >15% or evaluated as tertile change) and any outcome was observed (p-values between 0.08 – 0.92). No interaction between baseline levels or change of any of the markers and darbepoetin alpha on any outcome was observed (p-values for interaction 0.07-0.99).

Discussion

In 1582 patients with HFrEF and mild to moderate anemia, circulating markers reflecting monocyte/macrophage and T cell activation were associated with adverse outcomes following adjustment for a wide range conventional risk variables, including hsCRP, supporting a role for leukocyte activation in the progression of HF. However, the associations were markedly attenuated after further adjustment for hsTnT and NT-proBNP, limiting their clinical usefulness as biomarkers in HF. Darbepoetin alpha treatment increased MIF levels, but no interaction with baseline levels of any of the leukocyte markers and treatment on outcomes was observed.

The systemic immune activation in HF as reflected by high levels of various inflammatory cytokines in plasma or serum could at least partly reflect systemic activation of various leukocyte

subpopulations such as T cell and monocyte/macrophages. This activation of leukocyte subpopulations could again be mirrored by soluble markers such as those investigated in this study. Our evaluation of these markers with regard to long term stability (i.e. association between marker level and inclusion year) and bench stability, suggest they are robust markers with adequate pre-analytical characteristics. In addition to systemic inflammation, however, these markers could also reflect myocardial inflammation. Indeed, linear regression identified hsTnT as a strong predictor for most leukocyte markers. Since leukocyte activation and migration from the circulation to areas of myocardial inflammation may contribute to the tissue injury in chronic HF [3,6,5], circulating levels of these markers could therefore also reflect ongoing myocardial inflammation. Recently, Ptaszynska-Kopczynska found higher serum levels of sCD163 in 79 patients with HFrEF [22]. As for sIL-2R, we and others have reported increased circulating sIL-2R levels in HF [23,24], associated with a more aggressive clinical course [24]. Apart from these smaller studies, we could find no reports on our selected markers as predictors of adverse outcomes in large HF populations. However, we have previously shown in the present RED-HF population that although CRP is associated with poor prognosis following adjustment for conventional risk factors (i.e. step 1 in our adjustment strategy), it does not add information on top of NT-proBNP and TnT. Thus, our finding that high plasma levels of sCD163, MIF, sIL-2R and ALCAM were associated with the primary endpoint and all-cause mortality after adjustment for conventional risk factors and only modestly affected by adjustment for hsCRP supports that T cell and monocyte/macrophage activation markers may reflect ongoing myocardial inflammation and could be of importance in the progression of HF. However, the strong association between all leukocyte markers and NT-proBNP and hsTnT in RED-HF could contribute to the strong attenuation of risk in multivariable analysis when these established risk markers were included, limiting the clinical usefulness of soluble markers of T cell and monocyte/macrophage activation.

Patients with HF and diabetes have particularly poor outcome and increased inflammatory burden [12,25] as also observed for sCD163 and sIL-2R in diabetes patients in the present population. However, we found no evidence of increased risk in diabetic patients. In contrast, in patients without diabetes, high sCD163 levels were associated with enhanced risk of all-cause mortality, also in adjusted analysis. Consequently, enhanced immune activation, as reflected by the leukocyte markers assessed in the

present study, does not seem to confer additional risk of adverse outcomes in the presence of diabetes, at least in HF patients with anemia. Similarly, sIL-2R and ALCAM were also associated with ischemic etiology and sIL-2R with stroke and MI the last 6 months, which also carries an ischemic component. However, all markers were more strongly correlated with both NT-proBNP and TnT levels suggesting that myocardial inflammation per se is more impactful than that of ischemic etiology in patients with anemia and advanced chronic HF. Possibly, the burden of anemia masks relevant associations and our results cannot be generalized to HF patients without anemia or other inflammatory markers.

The cause of anemia in patients with HF may be related to an absolute or relative deficiency of, or resistance to, erythropoietin (EPO), to which inflammation may contribute through bone marrow suppression [26,27]. EPO administration has been shown to both reduce and augment inflammatory responses in circulating leukocytes [28-30]. Interestingly, darbepoetin alpha treatment was associated with an increase in MIF, which helps to sustain macrophage activation and is an important mediator of anemic complications and bone marrow suppression during malaria infection [31,32], and could therefore also contribute to resistance to EPO. Furthermore, in patients with chronic kidney disease, non-responders to EPO treatment are characterized by enhanced activation of both monocytes [33] and T cells [34]. Thus, although the RED-HF trial failed to detect a benefit of an erythropoiesis stimulating agent on clinical outcomes, leukocyte activation markers could be potentially identify patients that could have advantage of EPO treatment. However, no interaction between darbepoetin alpha treatment and baseline or change in levels during follow-up of any of the leukocyte markers on outcome was observed.

The strengths of the present investigation include the large number of patients studied with a high event rate and adjustment for multiple relevant confounders. On the other hand, a randomized trial may not necessarily reflect the "real-world" HF population and the use of composite endpoints has an inborn limitation. In addition, the trial populations had mild to moderate anemia, a high frequency of renal dysfunction and all markers were strongly influenced by race. The baseline association between circulating markers and outcome may therefore not apply to all HF patients. Finally, our interpretations are limited to our choice of markers, which represent less studied markers in the context of HF. Other markers of monocyte/macrophage activation such as neopterin and soluble CD14 have been associated with HF progression following ACS [35] and all-cause mortality in elderly [36] are possible candidates.

In conclusion, although our data support activation of monocytes/macrophages and T cells in the progression of HF, the lack of independence from hsTnT and NT-proBNP limits their clinical usefulness as prognostic markers in this population. However, our findings do not rule out that other leukocyte activation markers may prove useful.

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Figure legends

Figure 1. Association between baseline levels of leukocyte markers and all-cause mortality in the RED-HF cohort. Kaplan-Meier curves showing the cumulative incidence of all-cause mortality (n=1541) during the whole study (mean follow-up 2.3 years, range 0.0-6.0 years), according to tertiles at enrollment.

Figure 2. Association between baseline levels of leukocyte markers and (A) primary outcome or (B) all-cause death in the RED-HF study. Hazard ratios (HRs) and 95% confidence interval (CI) per SD change with P-values are shown for univariate (UNI) analysis, when adjusted for clinical and biochemical variables (S1), further adjusted for hsCRP (S2) and lastly for hsTnT and NT-proBNP (S3).

Table 1. Patient demographics

Characteristic	Sub-study (n=1588)
Age yrs, mean (SD)	70 (12)
Female sex, n (%)	685 (43)
Race (white/black), n (%)	1052/156 (66/10)
BMI kg/m^2, mean (SD)	27.1 (5.7)
NYHA (III or IV) n (%)	1057 (66)
Systolic BP, mean (SD)	120 (18)
Heart rate b.p.m., mean (SD)	72 (11)
Ischemic HF, n (%)	1137 (72)
Duration HF yrs, mean (SD)	5.4 (5.4)
LVEF %, mean (SD)	30.3 (6.8)
Medical history	
Hypertension, n (%)	1176 (74)
Diabetes, n (%)	710 (45)
Atrial fibrillation or flutter, n (%)	502 (32)
MI last 6 mo., n (%)	580 (37)
Medication	
ACE or ARB, n (%)	1428 (90)
Beta-blocker, n (%)	1351 (85)
Diuretic, n (%)	1452 (91)
Biochemistry	
Creatinine mg/dL, mean (SD)	1.5±0.6
eGFR mL/min/1.73m^2, mean (SD)	50±22
HbA1c %, mean (SD)	6.48±1.43
Hemoglobin g/dL, mean (SD)	11.0±0.7
Transferrin saturation %, mean (SD)	27.9±10.9
Platelets 10^9/L, mean (SD)	232±79
WBC 10^9/L, mean (SD)	6.7±2.2
hsCRP mg/L, median (IQR)	2.7 (1.1,6.8)
NT-proBNP pg/mL, median (IQR)	1823 (653,4244)
hsTnT ng/ml, median (IQR)	25.6 (15.0,42.8)

Patient characteristics are given as mean ± SD for continuous variables and % of cases for categorical variables. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; MI, myocardial infarction; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitive troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide

Table 2. Association between baseline levels of leukocyte markers (continuous) and the secondary composite outcome cardiovascular death in the RED-HF study. Hazard ratios (HRs) and 95% confidence interval (CI) per log change with P-values are shown for univariate (UNI) analysis, when adjusted for clinical and biochemical variables (S1), further adjusted for hsCRP (S2) and lastly for hsTnT and NT-proBNP (S3).

		Secondary Composite HR (95% CI) P-value	Cardiovascular Death HR (95% CI) P-value
sCD163	Uni	1.18 (1.07—1.30) 0.006	1.17 (1.05—1.31) 0.006
	S1	1.12 (1.01—1.23) 0.028	1.12 (1.01—1.26) 0.040
	S2	1.09 (0.98—1.20) 0.10	1.09 (0.98—1.23) 0.13
	S3	1.02 (0.93—1.12) 0.69	1.03 (0.92—1.16) 0.57
MIF	Uni	1.14 (1.06—1.23) 0.001	1.16 (1.07—1.27) 0.014
	S1	1.11 (1.02—1.20) 0.012	1.15 (1.05—1.26) 0.003
	S2	1.08 (0.99—1.17) 0.051	1.12 (1.02—1.23) 0.015
	S3	1.03 (0.94—1.12) 0.54	1.06 (0.96—1.17) 0.23
Granulysin	Uni	1.01 (0.92—1.12) 0.093	1.02 (0.91—1.14) 0.50
	S1	0.92 (0.84—1.01) 0.086	0.93 (0.83—1.04) 0.19
	S2	0.91 (0.83—1.00) 0.061	0.92 (0.82—1.03) 0.13
	S3	0.91 (0.83—1.00) 0.053	0.92 (0.82—1.02) 0.11
sIL-2R	Uni	1.34 (1.23—1.45) <0.001	1.29 (1.17—1.42) <0.001
	S1	1.14 (1.04—1.24) 0.005	1.09 (0.98—1.20) 0.12
	S2	1.11 (1.01—1.21) 0.027	1.05 (0.95—1.16) 0.35
	S3	1.00 (0.91—1.09) 0.98	0.96 (0.86—1.06) 0.41
ALCAM	Uni	1.17 (1.05—1.29) 0.033	1.16 (1.03—1.30) 0.059
	S1	1.07 (0.97—1.19) 0.19	1.08 (0.96—1.22) 0.22
	S2	1.06 (0.96—1.18) 0.27	1.07 (0.97—1.21) 0.30
	S3	0.99 (0.89—1.10) 0.81	1.00 (0.88—1.12) 0.95

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

Table 3. Serum levels of leukocyte markers during placebo and darbepoetin treatment in the RED-HF trial.

Leukocyte marker		Placebo		Darbepoetin		P value
		Levels	**Change**	**Levels**	**Change**	
sCD163 (μg/mL)	BL	0.78 (0.40, 0.99)	0.01	0.80 (0.42, 1.00)	-0.10	0.358
	3 mo.	0.79 (0.44, 0.96)	(-0.18, 0,22)	0.80 (0.44, 0.98)	(-0.20, 0.23)	
MIF (ng/mL)	BL	2.59 (1.13, 2.50)	0.01	2.51 (1.13, 2.45)	0.14	0.006
	3 mo.	2.67 (1.15, 2.35)	(-0.65, 0.61)	2.86 (1.18, 2.78)*	(-0.41, 0.81)	
GNLY (ng/mL)	BL	1.6 (1.0, 2.0)	0.0	1.6 (1.1, 1.9)	0.0	0.094
	3 mo.	1.6 (1.1, 2.0)	(-0.2, 0.2)	1.6 (1.1, 2.0)	(-0.2, 0.2)	
sIL2R (ng/mL)	BL	347 (188, 409)	2	355 (191, 409)	-7	0.199
	3 mo.	358 (180, 402)	(-51, 47)	335 (172, 396)*	(-50, 42)	
ALCAM (ng/mL)	BL	181 (134, 218)	0	186 (138, 222)	-3	0.146
	3 mo.	181 (135, 216)	(-25, 23)	182 (134, 216)	(-27.5, 23)	
hsCRP (mg/L)	BL	2.5 (1.0, 6.6)	0.1	2.9 (1.1, 7.1)	0.1	0.115
	6 mo.	2.6 (1.0, 7.0)	(-1.4, 2.2)	2.9 (1.3, 7.1)	(-1.6, 2.0)	
NT-proBNP (pg/mL)	BL	1799 (692, 4214)	6.0	1837 (586, 4262)	-36	0.943
	6 mo.	1706 (633, 3797)	(-536, 534)	1568 (462, 3774)*	(-659, 277)	
hsTnT (ng/mL)	BL	25.1 (14.8, 42.5)	0.4	26.4 (15.2, 42.8)	-0.1	0.351
	6 mo.	24.6 (14.7, 38.9)	(-4.4, 4.8)	24.9 (14.4, 40.4)	(-3.7, 4.1)	

Data are given as median and 25th-75th percentiles. *$p<0.05$ *vs.* baseline levels. ALCAM, activated leukocyte cell adhesion molecule; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitive troponin T; MIF, macrophage migration inhibitory factor; sCD163, soluble cluster of differentiation 163; sIL2R, soluble IL-2 receptor; NT-proBNP, N-terminal pro-brain natriuretic peptide