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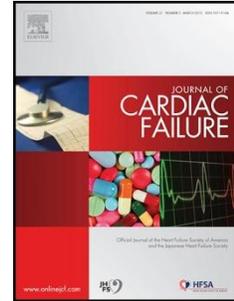
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Monocyte Subsets and Inflammatory Cytokines in Acute Decompensated Heart Failure

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Highlights

- Inflammation is known to be associated with heart failure.
- Decompensated heart failure (ADHF) patients had elevated inflammatory markers.
- ADHF patients had distinct monocyte subsets compared with healthy controls (HC).
- Treatment of heart failure made monocyte profiles more closely resemble that of HC.

ABSTRACT

Background: Distinct monocyte subsets predict cardiovascular risk and contribute to heart failure progression in murine models but have not been examined in clinical acute decompensated heart failure (ADHF).

Methods and Results: Blood samples were obtained from 11 healthy controls (HC) and at admission and discharge in 19 ADHF patients. Serological markers of inflammation were assessed on admission and discharge. Monocyte populations were defined using flow cytometry for cell-surface expression of CD14 and CD16: CD14⁺⁺CD16⁻ (classical), CD14⁺⁺CD16⁺ (intermediate), and CD14⁺CD16⁺⁺ (non-classical). In ADHF patients, C-reactive protein (CRP) and IL-6 were higher compared with HC (both $p < 0.001$), and decreased from admission to discharge (CRP: 12.1 ± 10.1 to 8.6 ± 8.4 mg/L, $p = 0.005$; IL-6: 19.8 ± 34.5 to 7.1 ± 4.7 pg/ml, $p = 0.08$). In ADHF patients, the admission proportion of CD14⁺⁺CD16⁻ monocytes was lower (68% vs. 85%, $p < 0.001$) and CD14⁺⁺CD16⁺ (15% vs. 8%, $p = 0.002$) and CD14⁺CD16⁺⁺ (17% vs. 7%, $p = 0.07$) monocytes higher compared with HC. Additionally, the proportion of CD14⁺⁺CD16⁻ monocytes increased (68% to 79%, $p = 0.04$) and the CD14⁺CD16⁺⁺ monocytes decreased (17% to 7%, $p = 0.049$) between admission and discharge.

Conclusions: Following standard treatment of ADHF, the monocyte profile and circulating inflammatory markers shifts to more closely resemble those of HC, suggesting a resolving acute inflammatory state. Functional studies are warranted to understand how specific monocyte subsets and systemic inflammation may contribute to ADHF pathophysiology.

Keywords: heart failure, innate immunity, monocytes, inflammation

Abbreviations: ADHF, acute decompensated heart failure; HC, healthy controls; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein

INTRODUCTION

Over 5 million Americans currently have heart failure (HF), and acute decompensated heart failure (ADHF) is the most common reason for hospital admission among persons older than 65.¹ With the increasing incidence of HF risk factors (e.g. age, obesity, and diabetes), ADHF hospitalizations are likely to increase in the coming years. Over one-third of patients who survive ADHF hospitalization are rehospitalized or die within 90 days of initial discharge², and 1-year mortality is over 30%³. Unfortunately, current therapy for ADHF is empiric and largely based on expert consensus.^{4,5}

Abnormalities in the inflammatory cascade have been associated with both the initiation and the progression of HF.⁶ The detrimental consequences of sustained inflammation in HF models led to several controlled trials aimed at neutralizing proinflammatory cytokines in HF patients. However, results were overall neutral in these studies and an appreciable number of patients had worsening of HF^{7,8}, raising important questions about how the immune system contributes to ADHF. The innate immune system could modulate the inflammatory component of HF through several mechanisms, including the production of inflammatory cytokines (e.g. TNF- α , IL-6) and reactive oxygen species, activation of the complement system, and modulation of endothelial cell function and myeloid cell recruitment.

Monocytes are key innate immune system mediators of inflammatory responses. Monocyte dysregulation has been implicated in the pathogenesis of diverse diseases including diabetes, tumor metastasis, pulmonary fibrosis, myocardial

infarction, and atherosclerosis. Functionally distinct monocyte subsets can be characterized using flow cytometry for surface expression of FcγIII receptor CD16 and the lipopolysaccharide receptor CD14. The Nomenclature Committee of the International Union of Immunological Societies defines three subsets by this method: CD14⁺⁺CD16⁻ (classical), CD14⁺⁺CD16⁺ (intermediate), and CD14⁺CD16⁺⁺ (non-classical).⁹ The distribution of these monocyte subsets changes with age,¹⁰ and affects the risk for cardiovascular events even if clinically evident vascular disease is not present.^{11, 12} Recently, Ismahil et. al. found that differential monocyte subpopulations causally contribute to disease pathogenesis in a murine model of HF,⁶ but data in human HF are sparse and cross-sectional.^{13, 14} We sought to identify changes in monocyte subsets over the course of HF hospitalization, as this information could suggest further avenues for the study of ADHF pathophysiology.

MATERIALS AND METHODS

Study design

The goals of this study were to compare serologic inflammatory markers and monocyte subsets between ADHF patients and healthy adult controls (HC), and to determine how these parameters change over the course of hospital treatment for ADHF. For the purposes of this study, three monocyte subsets were considered: CD14⁺⁺CD16⁻ (classical), typically accounting for 80-90% of cells in the circulation, and the CD14⁺⁺CD16⁺ (intermediate), and CD14⁺CD16⁺⁺ (non-classical) subsets, which account for the remaining 10-20%.⁹ Since changes in monocyte subsets during ADHF have not

previously been reported, and few studies have examined differences between HF patients and healthy controls, we powered the study based on IL-6, a marker of systemic inflammation. Based on a prior cross-sectional study of symptomatic HF outpatients vs. HC, we predicted a decrease in plasma IL-6 from 40 to 15 pg/ml between admission and discharge with standard deviation of ± 25 pg/ml and mean IL-6 of 3.0 pg/ml in HC. Using these assumptions, 20 or more ADHF subjects and 5 or more HC were believed sufficient to demonstrate significant between-group and pre-post ADHF hospitalization differences in plasma IL-6 levels at $\alpha = 0.05$ and $\beta = 0.20$.

Enrolled patients

Patients hospitalized at the University of Michigan Health System with an admitting diagnosis of ADHF were eligible for this study. Baseline exclusion criteria were acute coronary syndrome, severe renal dysfunction (dialysis or estimated glomerular filtration rate < 15 ml/min/1.73m²), cirrhosis or other evidence of severe hepatic dysfunction, active infection or inflammatory disorder (e.g. rheumatoid arthritis, lupus), active smoking, active solid organ or hematologic malignancy, use of immunosuppressive therapy (including corticosteroids), and planned mechanical circulatory support, cardiac transplantation, or other surgical procedure during the index hospitalization. Patients who were found to have active infectious or inflammatory conditions (e.g. pneumonia, gout flare) following initial enrollment and those who had surgical procedures, blood product transfusion, or immunosuppressive therapy during hospitalization were excluded from the analysis. Nonsmoking older adult (aged ≥ 65 years) HC were recruited through a database managed by the University of Michigan Claude D. Pepper Older Americans Independence Center.

Sample collection and analysis

ADHF patients with an anticipated hospital length of stay longer than 48 hours were enrolled and admission/baseline blood samples were drawn within 24 hours of hospital admission. Discharge blood samples were drawn on the date of hospital discharge, or within 24 hours prior to an anticipated weekend discharge according to laboratory facility availability. Blood samples were drawn from HC subjects at a single baseline visit.

Analyses for basic laboratory parameters including renal function, electrolytes, high-sensitivity C-reactive protein (hs-CRP), and complete blood count with differential were performed by the University of Michigan Pathology Laboratory using standard techniques. The University of Michigan Cancer and Immunology Core Laboratory measured plasma IL-6, IL-10, TNF- α , and TGF- β using commercially available high-sensitivity Luminex assays (EMD Millipore, Billerica, MA) and soluble CD-14, MCP-1, and RANTES levels using a commercially-available ELISA assay.

Monocyte isolation and flow cytometry

Monocyte populations were analyzed in all patients and assessed for expression of CD14 and CD16 to define the circulating monocyte populations. 8ml of whole blood was collected by venipuncture in Cell Preparation Tube (CPT) with Sodium Citrate (BD Vacutainer, Franklin Lake, NJ) for each study participant at the time points outlined above. The CPT

tubes were centrifuged at 2000xg for 30 min, inverted 5x, and stored overnight. The next day, the top layer was removed, split into 2 x 4ml aliquots, diluted up to 12 ml with ice cold PBS to wash, and centrifuged at 300xg for 10 minutes. After centrifugation, the supernatant was aspirated and the cell pellets were washed again with ice cold PBS. The cell pellet was resuspended in 2ml 1x RBX Lysis Buffer (Biolegend, San Diego, CA) and incubated for 10 minutes at room temperature in the dark. The RBC lysis buffer was neutralized with deionized water and then the cells were pelleted at 300xg and resuspended in Cell Staining Buffer (Biolegend, San Diego, CA). Cells were blocked for 10 minutes on ice with Human TruStain FcX™ (Fc Receptor Blocking Solution; Biolegend, San Diego, CA). Approximately 1×10^6 cells in 300ul of buffer were mixed with 15ul of antibody (Alexa Fluor® 647 anti-human CD14 Antibody #325611, Alexa Fluor® 488 anti-human CD16 Antibody #302022, Biolegend, San Diego, CA, or the appropriate isotype controls) diluted 1:200 in Cell Staining Buffer and incubated for 30 minutes in the dark on ice. Antibodies were titrated to determine optimal concentrations for staining. After incubation, the cells were washed two times with cold Cell Staining Buffer and analyzed using an Accuri C6 Flow Cytometer (Accuri Instruments, Inc., Ann Arbor, MI). At least 500,000 cells per sample were analyzed. Flow cytometry data was further analyzed using FlowJo software (Tree Star Inc., Ashland, OR). Dead cells were excluded using propidium iodide (PI) as previously described (ref). In all patients, the percent of dead cells as defined using PI was less than 5%. The percentage of CD14 and CD16 cells were determined in relation to the isotype control samples. Forward scatter height against forward scatter area was first determined to eliminate doublets. Then forward and side scatter were used to grossly define the monocyte population and then further stratified using CD14 and

CD16 to determine CD14⁺⁺CD16⁻ (classical), CD14⁺⁺CD16⁺ (intermediate), and the CD14⁺CD16⁺⁺ (non-classical) monocyte subsets as previously described.¹⁵ The analysis of monocyte subsets in peripheral blood mononuclear cell stains and the gating strategy used are depicted in Supplemental Figure 1. Populations were determined by gating on the CD14⁻ or CD14⁺ population and measuring the number of events in that population compared to total number of events collected. Percent positive after staining with Alexa Fluor® 647 anti-human CD14 or Alexa Fluor® 488 anti-human CD16 antibody for each sample was determined by gating on the CD14⁻ or CD14⁺ population in unstained cells, displaying the data as a histogram, and measuring the shift in the histogram above 5% after staining. Linear mixed-models analysis was used to estimate least squares means for monocyte population size and percent positive of each population after antibody staining (SAS 9.2; SAS Institute).

Statistical analysis

We used Wilcoxon signed-rank testing to evaluate differences in HF patients between hospital admission and discharge, including differences in proportion of monocyte subsets, and Mann-Whitney U testing to evaluate differences between HF patients and HC. GraphPad Prism 6 (GraphPad Software, La Jolla, CA) was used for between-group statistical comparisons.

RESULTS

Enrolled study population

A total of 28 ADHF patients were initially enrolled in the study; two were excluded from baseline analysis after enrollment due to lack of clinical ADHF or subsequently meeting exclusion criteria (< 24 hour length of stay with no clinical worsening from baseline in 1, biopsy-proven liver cirrhosis in 1). An additional seven HF patients were excluded from admission-discharge analysis (inpatient death in 1, left ventricular assist device scheduled or placed while inpatient in 3, patient withdrawal from study prior to hospital discharge in 2, and failure to obtain blood sample within discharge time window in 1). The baseline characteristics of the HC and ADHF patients are shown in **Table 1**. The ADHF patients were on average younger and as expected, had significant comorbidity burden.

Circulating cytokines and inflammatory biomarkers

On admission, ADHF patients had significant differences in levels of hs-CRP and IL-6 compared with HC (hs-CRP: 12.1 ± 10.1 vs. 0.6 ± 0.3 mg/L; IL-6 19.8 ± 34.5 vs. 1.7 ± 1.0 pg/ml, both $p < 0.001$). Additionally, in ADHF patients, hs-CRP significantly decreased from admission to discharge (hs-CRP: 8.6 ± 8.4 mg/L, $p = 0.005$; median change -1.1 mg/L, IQR of change -5.3 - 0.2 mg/L) and IL-6 trended lower (IL-6: 7.1 ± 4.7 pg/ml, $p = 0.08$; median change -2.6 pg/ml, IQR of change -14.2 - 1.7 pg/ml). IL-10, TGF- β , TNF- α , sCD-14, MCP-1, and RANTES levels did not significantly differ between ADHF patients and HC or between admission and discharge in ADHF (**Figure 1**).

Monocyte subsets

We observed differences in the distribution of monocyte subsets between ADHF patients upon hospital admission and HC. Specifically, the proportion of CD14⁺⁺/CD16⁻ (classical) monocytes was lower (68 vs. 85%, $p<0.001$) and that of CD14⁺⁺/CD16⁺ (intermediate) and CD14⁺/CD16⁺⁺ (non-classical) cells were higher (15 vs. 8%, $p=0.002$ and 17% vs. 7%, $p=0.07$, respectively) in ADHF patients at admission compared with HC. We also found that the monocyte subsets transitioned as ADHF patients were treated during their hospitalization. Compared with admission, we observed an increase in the CD14⁺⁺/CD16⁻ (classical) proportion (68 to 79%, $p=0.04$; median change 4%, IQR of change -3 to 21%) and decrease in the CD14⁺/CD16⁺⁺ (non-classical) proportion (17 to 7%, $p=0.049$; median change -2%, IQR of change -7 to 1%) upon hospital discharge, so that the overall monocyte subset distribution more closely resembled that observed in the HC (**Figure 2**). We found no correlations between baseline left ventricular ejection fraction, admission B-type natriuretic peptide, blood urea nitrogen, or serum sodium levels and baseline or changes in monocyte distribution. Similarly, we found no significant correlations between baseline or change in CRP, IL-6, MCP-1, or RANTES levels and baseline or changes in monocyte distribution.

In the ADHF group, 16 of 19 patients had total monocyte counts by automated differential available at both admission and discharge. Total monocyte count was higher in ADHF patients compared with HC, but did not change from admission to discharge in ADHF patients (ADHF admission: 0.6 ± 0.2 K/ μ L; ADHF discharge 0.7 ± 0.3 K/ μ L, $p=0.22$; HC: 0.4 ± 0.1 K/ μ L, $p<0.01$ vs. ADHF admission and ADHF discharge). Regardless if obtained at admission or discharge, all monocyte counts

in each subset were more numerous in ADHF patients than in HC ($p < 0.003$ for all). By total counts, the CD14⁺⁺/CD16⁻ (classical) subset significantly increased between admission and discharge in ADHF patients (0.02 ± 0.02 K/ μ L to 0.03 ± 0.02 K/ μ L, $p = 0.02$). Changes in the CD14⁺⁺CD16⁺ (intermediate) subset, and CD14⁺CD16⁺⁺ (non-classical) subsets by total counts were not significant ($p > 0.05$) between admission and discharge in ADHF; trends were in the same direction as for the subset proportions, but displayed substantial variability (**Figure 3**).

DISCUSSION

In this study, we demonstrate that 1) patients admitted with ADHF have serological evidence of inflammation (specifically, elevated levels of CRP and IL-6), 2) ADHF patients have a distinct monocyte subset profile compared with HC, and 3) following treatment of ADHF, the monocyte subset distribution of ADHF patients shifts to more closely resemble the HC monocyte profile. To our knowledge, this study is the first to explore how circulating monocyte subsets evolve over the course of ADHF treatment.

Recent basic science studies have implicated activation of myeloid cells (monocytes and macrophages) in the pathobiology of HF but few studies have investigated this issue in clinical HF.⁶ In one study of 30 ambulatory HF patients, absolute monocyte count increased with New York Heart Association class and predicted worsening HF or death over the subsequent 20 months. Furthermore, myeloid cells from these patients had higher CD11b and lower CXCR1 receptor

expression than healthy controls. A retrospective analysis from the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial demonstrated that absolute monocyte count predicted all-cause mortality and HF hospitalization on a univariate basis but not after adjusting for clinical variables. The authors hypothesized that specific monocyte subpopulations might predict HF outcomes better than absolute monocyte levels and called for prospective studies to address this and other related questions.¹⁶

The identification of distinct monocyte subsets in preclinical models provides a unique opportunity to investigate the immune system's role in human cardiovascular disease.¹⁷ CD16⁺ monocyte counts are increased in many inflammatory conditions, and are believed to modulate the inflammatory component of coronary artery disease. Even in patients without overt vascular disease, monocyte subsets carry important prognostic information for cardiovascular events. Rogacev and colleagues prospectively followed 119 patients with chronic kidney disease for an average of 4.9 years. They found that the CD14⁺⁺/CD16⁺ (intermediate) monocyte subset predicted combined death and major cardiovascular events (myocardial infarction, stroke, amputation, revascularization) independent of other risk factors, including prevalent vascular disease. The same group followed 951 patients referred for coronary angiography for a mean of 2.6 years in the HOM SWEET HOME (Heterogeneity of Monocytes in Subjects Who Undergo Elective Coronary Angiography—The Homburg Evaluation) study. Patients in the highest quartile of CD14⁺⁺/CD16⁺ (intermediate) monocyte count had three

times the risk of combined death and major cardiovascular events, even after extensive adjustment for overall leukocyte counts, traditional cardiovascular risk factors, and the presence of vascular disease at baseline.¹²

Only two prior reports have described monocyte subset distribution in human HF. In an outpatient cross-sectional study, Barisione and colleagues found that the proportions of CD14⁺⁺/CD16⁺ (intermediate) monocytes were increased and CD14⁺/CD16⁺ (non-classical) decreased in HF compared to controls without HF. The CD14⁺⁺/CD16⁺ (intermediate) proportion increased with New York Heart Association class, and was inversely related to left ventricular ejection fraction. In another cross-sectional study, Amir et. al also reported decreased proportion of CD14⁺/CD16⁺ (non-classical) monocytes in patients with stable HF, but found no difference in CD14⁺⁺/CD16⁺ (intermediate) cells when compared to age-matched controls.¹⁴ In contrast to both studies in chronic HF patients, we found that ADHF patients upon hospital admission had substantially higher proportion of CD14⁺/CD16⁺ (non-classical) monocytes than HC; this proportion then decreased over the course of hospitalization to more closely resemble that found in HC. To our knowledge, our study is the first to report monocyte subset distribution in ADHF and changes during hospitalization. The novel aspect of our findings is that monocyte subsets in ADHF patients evolved over a short timeframe during standard inpatient treatment, despite no presence of infection or use of direct immune-modulating therapies. This observation suggests that the immune system acutely responds to both HF decompensation and its treatment.

This concept is supported by the work of Colombo and colleagues, who suggest that vascular inflammation is a key factor in ADHF.¹⁸ They first demonstrated that severely ill ADHF patients requiring inotropic support had increased venous endothelial expression of pro-inflammatory genes that improved following clinical re-compensation.¹⁹ A subsequent study in healthy young people showed that venous congestion substantially alters endothelial gene expression in several relevant domains, including leukocyte adhesion molecules (VCAM-1) and chemokines (CXCL-2, secreted by monocyte/macrophage lineage cells).²⁰

Several studies have demonstrated immune-modulating effects of commonly used HF medications including angiotensin-converting enzyme inhibitors and beta blockers.^{21, 22} Recently, genetic ablation of the mineralocorticoid receptor, the molecular target for spironolactone and eplerenone, in macrophages and endothelial cells demonstrated a profound impact on cardiac remodeling in preclinical models of HF.²³ These studies provide possible mechanistic links to the circulating monocyte findings in our study. However, these chronic HF medications did not change substantially during hospitalization (see *Supplementary Table 1*) and would be unlikely to explain the monocyte alterations observed over the short-time frame of ADHF treatment. The vast majority of ADHF patients worldwide are treated with intravenous loop diuretics to remove volume overload, including all ADHF patients in our study. Failure to resolve symptomatic congestion during ADHF hospitalization increases the risk for rehospitalization and death,²⁴ but high doses of diuretics and worsening renal function also predict poor outcomes.²⁵ Further investigation of the relationship between monocyte phenotypes,

endothelial activation, heart failure therapeutics, and volume status may clarify the mechanisms through which the immune system modulates ADHF and suggest new treatment approaches.

Similar studies in obesity and trauma demonstrate that monocyte subsets have prognostic value with regards to disease severity and clinical outcomes^{26, 27}. Importantly, in all of these diseases, our current understanding has progressed first from an appreciation that absolute monocyte levels are predictive, to a more nuanced appreciation that monocyte heterogeneity and ultimately the functional consequences of this heterogeneity contribute to disease pathogenesis. Recent preclinical studies have demonstrated causal contributions of circulating myeloid cells to HF directly implicating these cells and the systemic inflammatory response to HF pathogenesis.⁶ Interestingly, the authors found that transfer of immune cells from animals with HF recapitulated multiple facets of cardiac remodeling in normal mice again supporting the notion that circulating immune cells modulate cardiac responses present in HF. We believe our hypothesis-generating results from clinical HF populations support these notions and further contribute to the expanding body of knowledge linking immune dysfunction and HF.

Limitations

This pilot study includes a small number of patients, and we were unable to examine the relationships between monocyte profiles and clinical variables or outcomes. The HC group was slightly, but not significantly, older than the ADHF cohort.

However, healthy aging skews monocytes towards CD16+ subsets, and accordingly this difference should have biased towards no difference in monocyte profiles between ADHF patients and HC. Additionally, we also were unable to obtain total monocyte counts at both admission and discharge in three ADHF patients. The data were analyzed both by excluding those patients as well as by carrying over the single available monocyte count (from admission or discharge) with no obvious change in the reported trends.

The ADHF patient cohort we analyzed was quite advanced, with low blood pressure, markedly elevated B-type natriuretic peptide levels, nearly half not tolerating angiotensin-converting enzyme inhibitor or angiotensin receptor blockade at hospital discharge, and requiring high loop diuretic doses for clinical stability. Whether our findings would apply to a less ill ADHF population is unknown. As mentioned above, monocyte distribution did not appear related to circulating cytokines/chemokines or several clinical markers of HF severity. Our results suggest that functional assessment of monocytes may help clarify the relationships between traditional markers of HF severity and the innate immune system.

Finally, there exist many methodologies to characterize circulating monocyte populations and these methodologies are rapidly evolving along with our appreciation of the tremendous plasticity of monocyte populations^{9, 17, 28}. Over the recent years, considerable effort has been dedicated to more accurately define monocyte subsets and to ascribe functional significance to these flow cytometric-based classifications. For the purpose of this exploratory study, we focused primarily

on morphological characteristics of peripheral blood mononuclear cells to identify monocyte populations and then used flow cytometry of cell surface markers to further define the monocyte subsets. There exist many methodological approaches to characterize monocyte subsets. As with all approaches, there is always the possibility of contamination by other circulating immune cells (neutrophils, NK cells) in our monocyte populations. The small contamination from these other circulating cells would be more problematic if our analysis included genomic profiling which can amplify transcripts from small populations of cells; however, because we focused on the cell-surface markers and how these evolve with standard HF treatment, we do not believe these will fundamentally skew the conclusions presented here. Studies are already underway to further characterize these monocyte populations using FACS sorting and purification to allow for more precise genomic and proteomic characterization of these circulating monocyte populations.

CONCLUSION

In this study of patients hospitalized for ADHF, serological markers of inflammation were elevated and monocyte subset distribution substantially differed from HC. Following standard ADHF treatment, CD14+/CD16++ (non-classical) monocytes decreased and CD14++/CD16- (classical) monocytes increased so that the overall subset profile more closely resembled that of the HC. Further studies are warranted to clarify the relationship between circulating monocyte subsets and ADHF pathophysiology.

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ABBREVIATIONS

ADHF, acute decompensated heart failure; HC, healthy controls; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein

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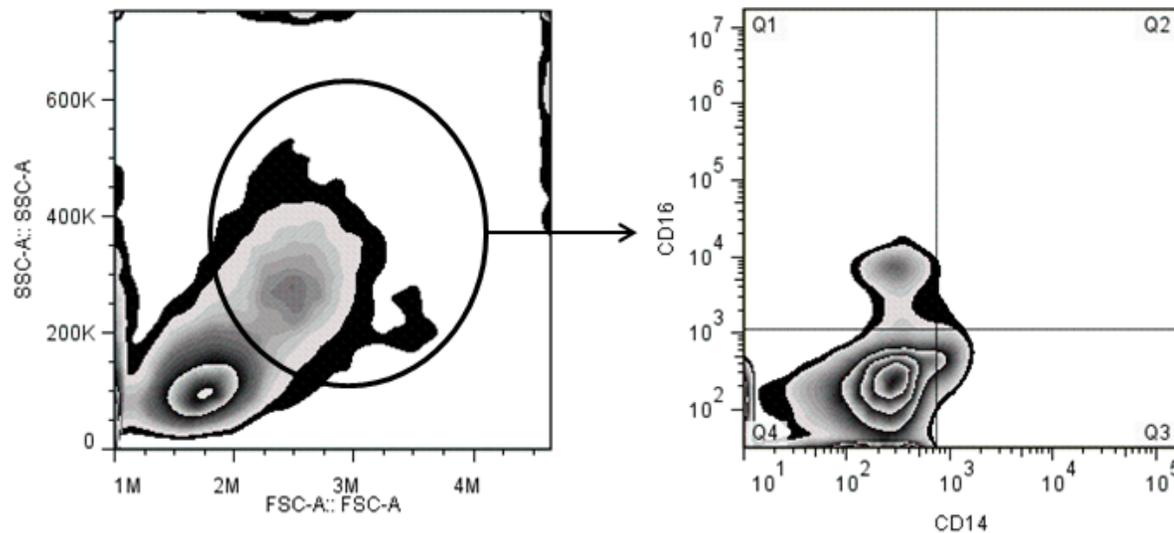
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Supplemental Figure 1. Representative flow cytometry dot plots demonstrating gating strategy employed to define monocyte subsets. A) Live gate for total leukocytes based on the forward scatter (FSC) and side scatter (SSC) properties. B) Separation of monocyte populations based on CD14 and CD16 expression.

Supplementary Table 1: Medication use at admission and discharge in heart failure patients

Medication type	% taking on admission	% taking on discharge
Beta-blocker	100%	89%
ACE/ARB	68%	53%
Spironolactone	58%	63%
Hydralazine	11%	26%
Isosorbide mononitrate	11%	26%
Thiazide-type diuretic	26%	32%
Loop diuretic	95%	100%
Furosemide equivalent daily dose (in mg)	187 ± 171	220 ± 151

FIGURE LEGENDS**Figure 1: Cytokines and Biomarkers**

Abbreviations: HC, healthy controls; HF, heart failure

* $p < 0.05$ HF admit vs. HC; † $p < 0.05$ HF discharge vs. admit

Figure 2: Monocyte Subset Distribution

Abbreviations: HC, healthy controls; HF, heart failure

* $p < 0.05$, HF vs. HC; † $p \leq 0.05$, HF discharge vs. admit

Figure 3: Total Monocyte Count

Abbreviations: HC, healthy controls; HF, heart failure

† $p < 0.05$ HF discharge vs. admit; all HF vs. HC comparisons $p < 0.003$

Figure 1: Cytokines and Biomarkers

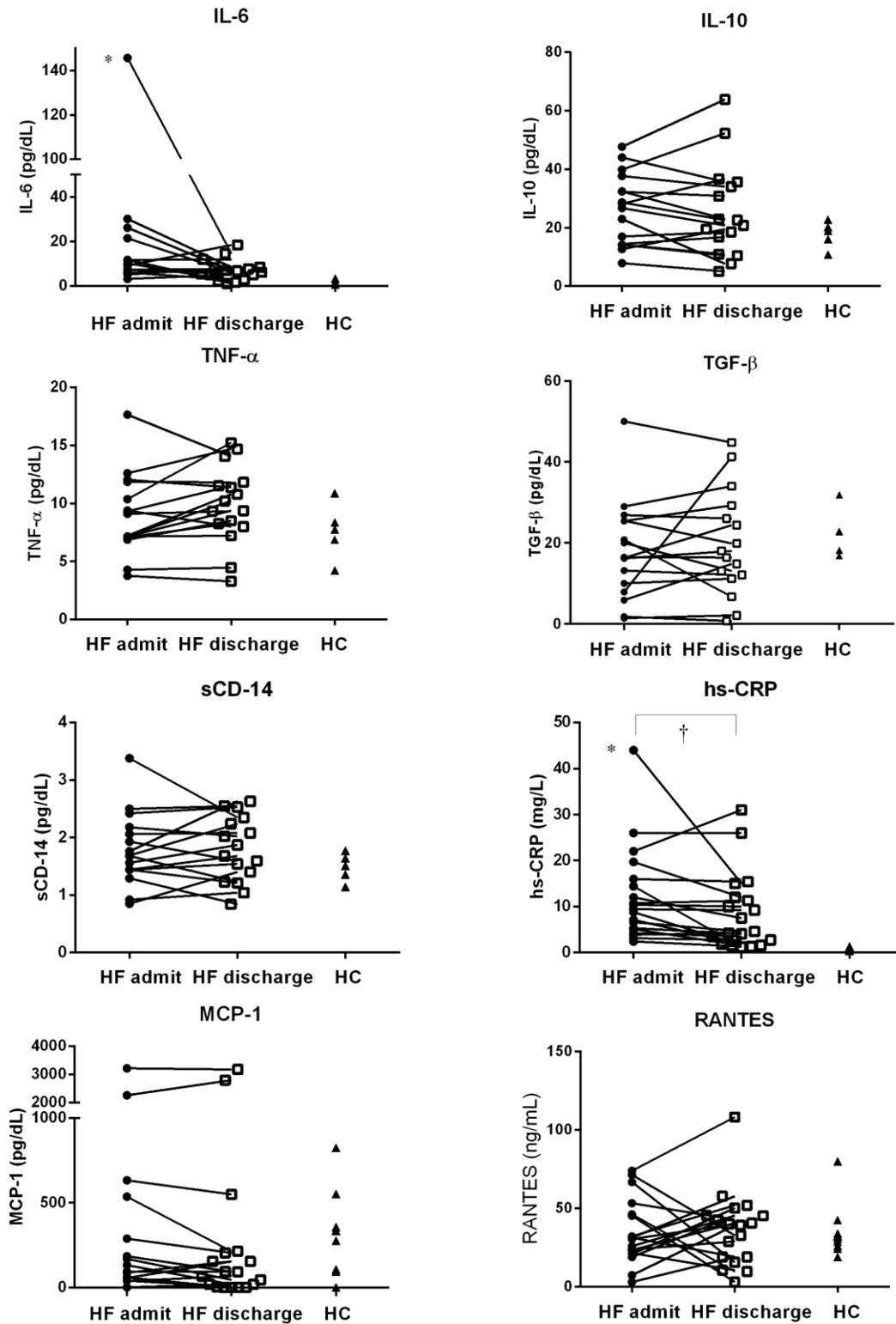


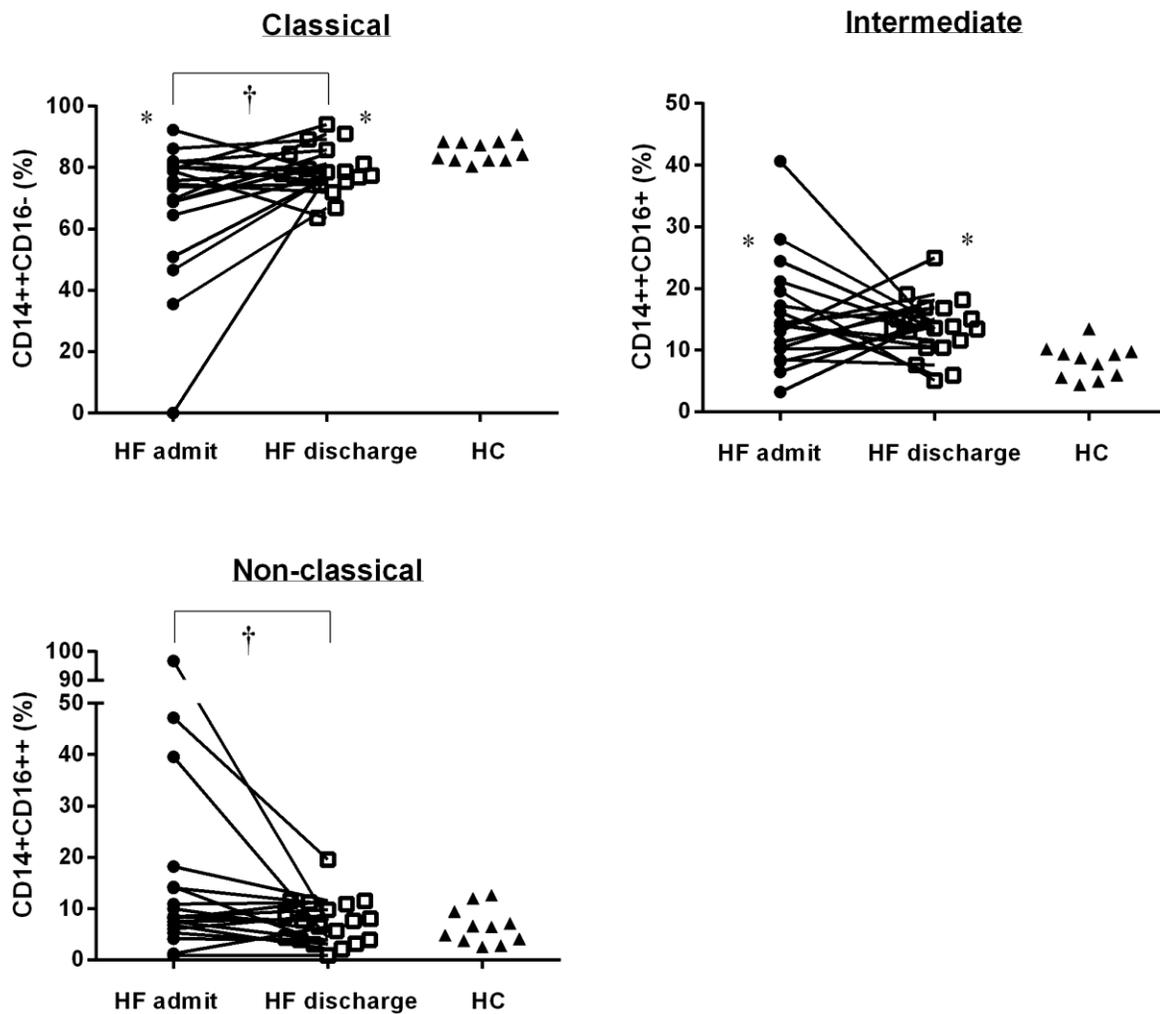
Figure 2: Monocyte Subset Distribution

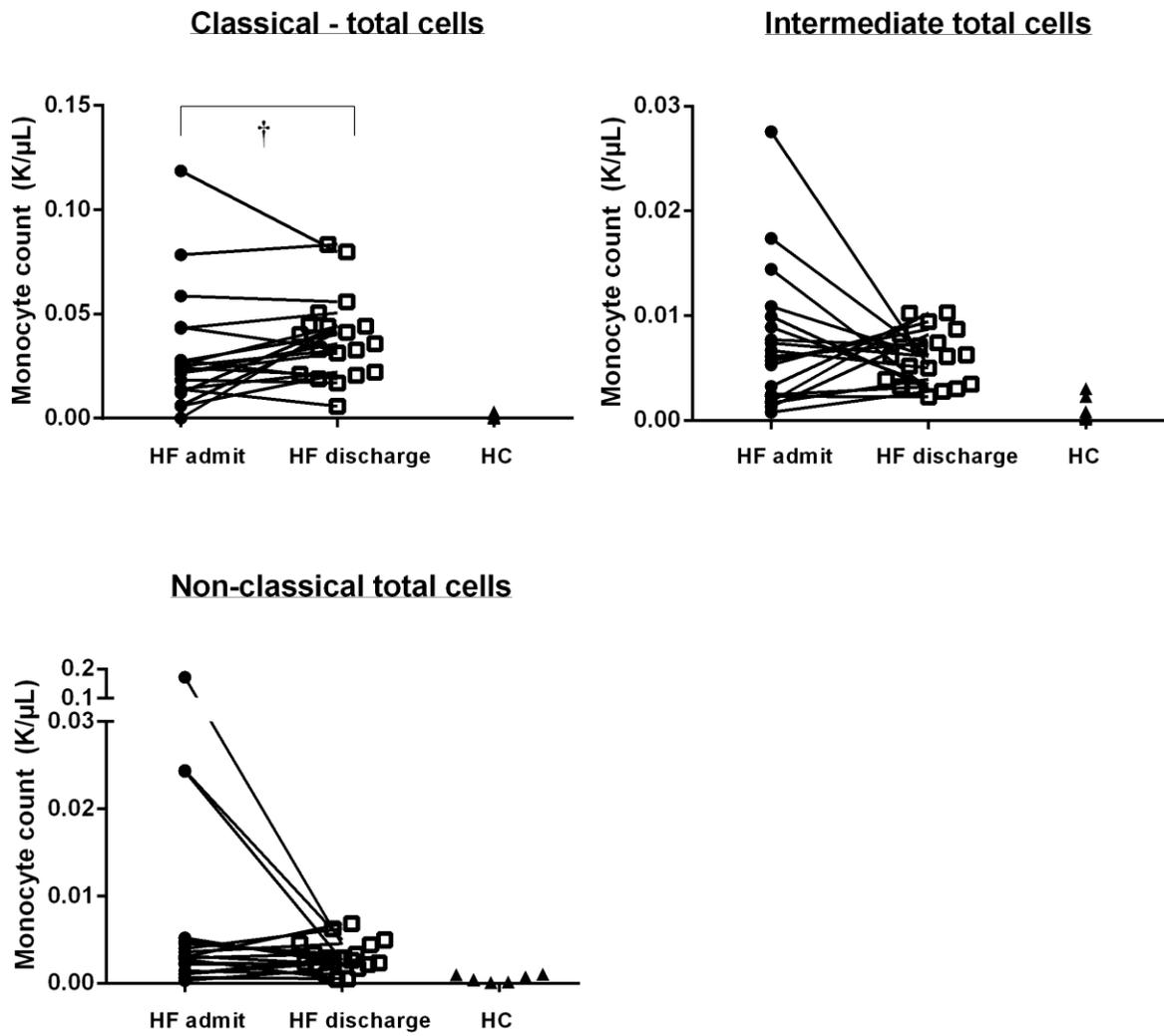
Figure 3: Total Monocyte Count

Table 1. Baseline clinical characteristics

Variables	HF patients (n = 19)	Healthy controls (n = 11)
Age, years	56 ± 11	60 ± 16
Female	4 (21%)	3 (27%)
Body mass index (kg/m ²)	34 ± 6	25 ± 4*
Ejection fraction, %	20 ± 15	n/a
<i>History of:</i>		
Coronary disease	8 (47%)	0*
Hypertension	11 (65%)	0*
Chronic kidney disease	10 (59%)	0*
Diabetes mellitus	11 (65%)	0*
<i>Admission/baseline:</i>		
Systolic blood pressure, mmHg	112 ± 18	129 ± 12*
WBC count (K/μl)	7.4 ± 1.9	5.7 ± 1.3*
Monocyte count (K/μl)	0.6 ± 0.2	0.4 ± 0.1*
Hemoglobin, g/dl	12.5 ± 3.5	13.8 ± 0.9*
Urea nitrogen, mg/dl	36 ± 24	18 ± 5*
Serum creatinine, mg/dl	1.4 ± 0.5	0.9 ± 0.2*
Estimated GFR† (ml/min/1.73m ²)	58 ± 22*	80 ± 25*
B-type natriuretic peptide (pg/ml)	994 ± 665	

Abbreviations: GFR, glomerular filtration rate; HF, heart failure; WBC, white blood cell

* p<0.05 between HF patient and healthy control groups

† Using Modification of Diet in Renal Disease equation

Data are presented as mean ± standard deviation.

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