Increased Formation of Monocyte-Platelet Aggregates in Ischemic Heart Failure

Benjamin J. Wrigley; Eduard Shantsila, PhD; Luke D. Tapp; Gregory Y.H. Lip, MD

Background—Cross-talk between monocytes and platelets is reflected by the formation of monocyte-platelet aggregates (MPAs). It is not known whether MPAs are affected in heart failure (HF), and we examined differences in patients with acute HF (AHF), stable HF (SHF), stable coronary artery disease (CAD) without HF, and healthy controls (HCs).

Methods and Results—MPAs were analyzed by flow cytometry for the 3 monocyte subsets (CD14++CD16-CCR2+ [Mon1], CD14++CD16+CCR2+ [Mon2] and CD14+CD16++CCR2– [Mon3]) in patients with AHF (n=51), SHF (n=42), stable CAD (n=44), and HCs (n=40). Counts of total MPA and MPAs associated with Mon1 and Mon2 were significantly higher in AHF compared with SHF, CAD, and HCs (P<0.001 for all). The proportion of Mon1 aggregated with platelets was increased in AHF compared with SHF (P=0.033), CAD (P<0.001), and HCs (P<0.001). A higher percentage of Mon3 aggregated with platelets was also seen in AHF compared with SHF (P=0.012) and HCs (P<0.001) but not compared with CAD (P=0.647). MPAs associated with Mon2 were significantly lower in patients who experienced adverse clinical outcomes of death or rehospitalization compared with those who remained free of events (P=0.03). Mon2 count remained an independent negative predictor of combined death and rehospitalization after adjustment for age, left ventricular ejection fraction, creatinine, and brain natriuretic peptide (hazard ratio, 0.58 [95% CI, 0.34–0.98]; P=0.043).

Conclusions—MPA formation in patients with both acute and stable HF is increased and seems to be confined to monocytes from Mon1 and Mon2 subsets. MPAs associated with Mon2 seem to be negatively predictive of a worse prognosis in AHF. (Circ Heart Fail. 2013;6:127-135.)

Key Words: heart failure ■ inflammation ■ monocytes ■ monocyte-platelet aggregates ■ thrombosis

Systolic heart failure (HF) is both an inflammatory and prothrombotic condition, as evidenced by elevated levels of circulating cytokines and an increased risk of thromboembolic events.1 Platelets are the major cellular component of thrombosis,2 and patients with HF have enhanced platelet activation, as reflected by increased whole blood aggregation,3 high mean platelet volume,2 and increased platelet P-selectin surface exposure.4 In addition to roles in hemostasis and thrombosis, platelets are also able to regulate the activity of other cell types, and cross-talk between monocytes and platelets is reflected by formation of monocyte-platelet aggregates (MPAs). MPAs have been shown to be a more sensitive marker of platelet activation than platelet surface P-selectin, because degranulated platelets rapidly shed P-selectin but still function in the circulation.6,7 Monocyte aggregation with platelets is accompanied by monocyte activation, resulting in increased cytokine production, expression of cell-adhesion molecules, and the release of matrix metalloproteinases, all of which may be important in collagen breakdown and left ventricular (LV) dysfunction.8

Even in the absence of platelet interaction, monocytes play an important role in inflammation and thrombosis, performing vital functions such as phagocytosis, cytokine production, and tissue repair,9 as well as being a major source of blood tissue factor.10 The specific role of monocytes in HF is largely unknown, although monocyte levels are increased in patients who develop LV dysfunction after myocardial infarction (MI), and a recent study also demonstrated increased monocyte numbers in patients with stable systolic HF.11 Furthermore, monocytes are heterogeneous and consist of distinct subpopulations with differing phenotype and functional characteristics.12,13 These subsets can be defined by flow cytometry according to differential expression of surface markers, giving rise to classic CD14++CD16–CCR2+ (Mon1), intermediate CD14++CD16+CCR2+ (Mon2), and nonclassic CD14+CD16++CCR2– (Mon3) subsets.13

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Although data on these subsets in HF are limited, the Mon2 subset has a distinct gene expression profile compared with the other subsets and particularly express genes linked to inflammation and angiogenesis, which may be important in tissue remodeling.14 These findings are also in accordance with recent data demonstrating high expression of cell surface receptors associated with angiogenesis and tissue repair on Mon2, as well as their abundance in bone marrow.15

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Correspondence to Eduard Shantsila, PhD, University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham B18 7QH, United Kingdom. E-mail: e.shantsila@bham.ac.uk
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MPAs are increased in numerous conditions associated with ischemia and thrombosis, such as acute MI, limb ischemia, and stroke. However, an interaction between monocytes (and their individual subsets) and platelets has not been studied in HF, and the possible impact on clinical outcome is unknown.

In this study, we aimed to compare levels of MPAs in patients with acute HF (AHF), stable HF (SHF), and control subjects with normal LV function, as well as dynamics of monocyte-platelet interactions and their association with clinical outcomes in patients with AHF.

Methods

Cross-Sectional Study

Fifty-one consecutive patients admitted to hospital with ischemic systolic AHF were recruited. AHF was defined in accordance with the European Society of Cardiology guidelines as the rapid onset/progression of HF symptoms and signs secondary to abnormal cardiac function requiring hospital admission. All of the AHF patients had documented LV ejection fraction (LVEF) ≤40% on echocardiography or left ventriculography with New York Heart Failure Association class IV on admission. Patients presenting with an acute coronary syndrome were excluded. Patients with AHF were compared with the following: (1) 42 subjects with ischemic SHF (LVEF ≤40% and no deterioration in clinical status, hospital admission, or change in medication during the preceding 6 months); (2) 44 patients with stable coronary artery disease (CAD), having MI >6 months before the recruitment or angiographically documented coronary artery stenosis >50% with LVEF >50%; and (3) 40 healthy controls (HCs) free from clinical disorders, cancer, hemodynamically significant valvular heart disease, atrial fibrillation, renal failure [creatinine >200 μmol/L], steroids, and hormone replacement therapy. Recruitment for all 4 of the study groups was undertaken during an 18-month period (October 30, 2009, to April 30, 2011), and patients were selected from 2 hospital trusts within the West Midlands, United Kingdom.

Nonfasting peripheral venous blood samples were obtained from all of the study subjects with a 21-gauge needle with minimal stasis into commercial tubes (Greiner Bio-One, Gloucestershire, United Kingdom) containing EDTA (for flow cytometric analysis) and sodium citrate (for platelet marker CD42a (glycoprotein IX) detection and accurately enumerate the 3 monocyte subsets and their aggregates with platelets. The technique is robust and highly reproducible, and the laboratory coefficient of variation for absolute monocyte count and MPA is 1.9%.

Mouse antihuman monoclonal fluorochrome-conjugated antibodies anti-CD16-Alexa Fluor 488 (clone DJ130c, AbD Serotec, Oxford, United Kingdom), anti-CD14-PE (clone MPHA9, BD), and anti-CCR2-APC (clone 48607, BD Systems Europe Ltd, Abingdon, United Kingdom) were used according to the manufacturer’s recommendations. MPAs were defined as CD14++CD16−CCR2+ (classic, Mon1), CD14++CD16+CCR2+ (intermediate, Mon2), and CD14+CD16+CCR2− (nonclassic, Mon3) monocytes. Absolute counts of monocyte subsets (in cells per microliter) were obtained by calculating the number of monocytes proportional to the number of the count beads according to the manufacturer’s recommendations. MPAs were defined as events positive to both monocyte markers (as above) and the platelet marker CD42a (glycoprotein IX). The number of events collected was ≥400 events for each monocyte subset and 10000 count beads.

Plasma Markers

To investigate correlations between MPA and markers of systemic inflammation and monocyte recruitment, plasma levels of interleukin 6 (IL-6) and monocyte-chemoattractant protein 1 (MCP-1) were measured by cytometric bead array technology. The BD FACSCalibur flow cytometer was used for data acquisition, with FCAP Array version 2.0.2 software (Burnsville, MN) for data analysis. Commercially available Human IL-6 Flex Set and Human MCP-1 Flex Set (both from BD) were used according to the manufacturer’s recommendations, as reported previously. The lower limits of detection were 1.0 pg/mL for IL-6 and 1.3 pg/mL for MCP-1. Brain natriuretic peptide (BNP) was measured using a commercially available enzyme immunoassay set (human BNP-32, Peninsula Laboratories, LLC, San Carlos, CA) according to the manufacturer’s specifications. The interassay and intra-assay coefficients of variation for all of the assays was <5%.

Power Calculation

Based on our previous work, the calculated (based on ANOVA) minimum number of participants in each group required to detect an assumption of minimal difference of 0.5 SD in the count of MPA between the study groups with 80% power (1-β=0.8) with α=0.05 (2-tailed) was n=35.13

Statistical Analysis

Values were expressed as mean (SD), median (interquartile range [IQR]), or percentages, for normally distributed, nonnormally distributed, and categorical variables, respectively. Cross-sectional comparisons among the 4 study populations were made using a χ² test (for categorical variables) and 1-way ANOVA and Kruskal-Wallis test for normally distributed and nonnormally distributed measures, respectively. A post hoc Tukey test was performed to assess intergroup differences, where appropriate. Arithmetical transformation was performed on nonnormally distributed variables before post hoc analysis. Repeated-measures ANOVA for normally distributed data and Friedman test for nonnormally distributed data were used to analyze longitudinal changes in study parameters. For patients with AHF, correlation coefficients were calculated by Pearson and Spearman tests for normally and nonnormally distributed data, respectively. Univariate and multivariate Cox regression analyses were used to determine predictors of the study end point. For multivariate analysis we included predictors identified in the univariate test. Recognizing the relatively small sample size, variables achieving P<0.10 on univariate testing were entered into a multivariate Cox regression analysis. Age was also included in this analysis because of its well-recognised association with mortality. Kaplan–Meier estimates for the distribution of time from index
admission to the primary end point were computed, and log-rank analysis was performed to compare event-free survival according to quartiles of MPAs with Mon2. Data analysis was carried out using SPSS 18.0 (SPSS Inc, Chicago, IL) with a $P$ value <0.05 considered statistically significant.

**Results**

**Cross-Sectional Analysis**

Relevant demographic and clinical characteristics are summarized in Table 1. Patients with HF had higher creatinine values than controls ($P<0.001$), and HCs included fewer cigarette smokers compared with other groups ($P<0.001$)

**Monocyte-Platelet Aggregates**

The total MPA count and MPAs associated with Mon1 and Mon2 were significantly increased in the AHF group compared with the 3 control groups (Table 2 and Figure 2). MPAs associated with Mon3 were higher in the AHF group compared with HCs ($P<0.001$) but similar to SHF and CAD groups.

The proportion of Mon1 aggregated with platelets was increased in AHF compared with SHF ($P=0.03$), CAD ($P<0.001$), and HCs ($P<0.001$). There were no differences in the proportion of Mon2 aggregated with platelets across the study groups. A higher percentage of Mon3 aggregated with platelets.
was seen in the AHF group compared with SHF \(P=0.012\) and HCs \(P<0.001\) but not compared with CAD \(P=0.22\).

Patients with SHF had significantly higher total MPA count compared with HCs \(P=0.02\), and there was a trend toward higher counts compared with CAD \(P=0.07\). Similarly, MPAs associated with Mon1 were significantly higher in patients with SHF compared with HCs \(P<0.001\), but there were no differences when compared with the CAD group \(P=0.10\). MPAs associated with Mon2 were higher in the SHF group compared with CAD \(P=0.04\) and HCs \(P=0.004\). There were no differences between MPAs associated Mon3 when comparing SHF with CAD and HCs.

The proportion of Mon1 aggregated with platelets was higher in the SHF group compared with HC \(P=0.01\) but was not different compared with CAD \(P=0.59\). The proportion of Mon2 and Mon3 aggregated with platelets did not differ among SHF, CAD, and HCs.

### Longitudinal Analysis

Fifty-one patients admitted with AHF were recruited to the study. Medications did not change during the study period, and specifically, 80% of patients were taking an antiplatelet drug (aspirin, clopidogrel, or both) at the first time point when blood was taken, with no changes made during the follow-up period. The median length of hospital stay was 8.5 days (IQR, 5.0–12.8 days). None of the patients experienced MI or stroke during follow up, but 20 patients (39.2%) experienced an adverse clinical event during follow-up (15 patients [29.4%] died, and an additional 5 patients [9.8%] were readmitted to the hospital with HF), with a median time to event of 129 days (IQR, 70–209 days). Of those who died, 13 patients died of HF, 1 died from a ruptured abdominal aortic aneurysm, and the cause of death in 1 patient was unknown. The remaining patients were followed up for a median of 387 days (IQR, 223–550 days).

Thirty-six AHF patients (71%) completed all 3 of the blood test time points and were used to analyze changes in MPA parameters over time. For those not completing all 3 of the blood tests, 11 had already reached a clinical end point (death or rehospitalization) and 4 were lost to follow-up but had not reached the end point according to medical charts. When compared with measurements taken during the first 24 hours of admission, the total MPA count and MPAs associated with...
Monocyte-Platelet Aggregates as Predictors of Clinical Outcome

All 51 of the AHF patients were included in the analysis to look at predictors of clinical outcome. MPAs associated with Mon2 (measured during the first 24 hours after hospital admission) were significantly lower in patients who experienced adverse clinical outcomes of death or rehospitalization (12.8 cells/µL; IQR, 7.1–19.1) compared with those who remained free of events (16.1 cells/µL; IQR, 11.2–27.7; P=0.03). The total MPA count and levels of MPAs with Mon1 and Mon3 did not differ significantly between patients with or without events.

In a univariate Cox regression analysis, BNP (P=0.009), creatinine (P=0.013), and MPA associated with Mon2 (P=0.042) were predictors of clinical outcome, with LVEF showing a strong trend (P=0.061; Table 4). In a multivariate Cox regression analysis, Mon2 count remained an independent negative predictor of combined death and rehospitalization after adjustment for age, LVEF, creatinine, and BNP (Table 4).

For Kaplan–Meier analysis, patients were grouped by quartiles of MPAs associated with Mon2 (Figure 3): (1) quartile 1, ≤10 cells/µL; (2) quartile 2, >10 to 16 cells/µL; (3) quartile 3, >16 to 24 cells/µL; and (4) quartile 4, >24 cells/µL. Patients with the lowest counts of MPAs from Mon2 (quartile 1) had significantly worse clinical outcome compared with those with the highest counts (quartile 4; log rank test; P=0.037).

Correlation Analysis of MPAs With Plasma Markers and LVEF

In patients admitted with AHF, plasma concentration of IL-6, MCP-1, and BNP were 11 (7–16), 125 (7–16), and 24 (12–36) pg/mL, respectively. Table 2 presents the cross-sectional analysis of MPAs in the study groups.
aggregation; Mon1, CD14+CD16– monocytes; Mon2, CD14++CD16+CCR2+ monocytes; and Mon3, CD14+CD16++CCR2– monocytes.

† Data show admission vs follow-up, P=0.009.
‡ Data show admission vs follow-up, P=0.003.
\[P\, Value\]

Discussion

MPAs represent a sensitive marker of both monocyte and platelet activation, and we have shown for the first time that the total MPA count is increased in patients with both AHF and SHF compared with controls with normal LV contractility. Importantly, increased formation of MPAs in AHF is confined to monocytes from the Mon1 and Mon2 subsets and not from the Mon3 subset. We have also shown that MPAs from Mon2 are inversely predictive of adverse outcome in patients admitted to hospital with AHF, independent of age, LVEF, and renal function. The cause of death in the majority of patients was because of worsening of HF, and no patients experienced stroke or MI during follow-up. Thus, an association between clinical outcome and the formation of MPAs seems to be in addition to the acknowledged increased risk of thromboembolism in patients with HF.

It remains unclear why MPAs from Mon2 are increased in AHF compared with controls, and yet patients who have the worst outcomes have the lowest numbers of such complexes. One might speculate that MPAs from this subset of monocytes have beneficial or reparative properties in the myocardium, and a failure to form MPAs in AHF results in a greater insult to the myocardium and, hence, a worse prognosis. Alternatively, circulating MPA levels may not reflect their abundance within the myocardium itself, and low levels of circulating MPAs from Mon2 in those patients who have the worst prognosis may reflect a rapid migration and uptake of these complexes into the myocardium. Indeed, the transendothelial migration of MPAs is associated with a dissociation of the aggregate.\(^{19}\) Nonetheless, the observational nature of this study does not allow mechanistic insights into the specific roles of MPAs in HF, and further data on the prognostic role of MPAs are required.

Mon2 monocytes are increased in inflammatory conditions, and the formation of MPAs may reflect another marker of their proinflammatory activity. In acute coronary syndrome, MPAs represent a link between inflammation and thrombosis, with increased numbers formed in patients with troponin-positive coronary events, perhaps reflecting instability of coronary plaques and vascular inflammation.\(^{20–22}\) In HF, the precise trigger for MPA formation is less clear, although contributing factors are likely to include haemodynamic changes, activation of the renin-angiotensin system, endothelial dysfunction, and increased catecholamines.\(^{21}\)

As well as simply representing a marker of platelet activation, a recent study has shown that the formation of MPAs results in significant upregulation of circulating CD16\(^+\) monocytes (mainly Mon2), which adhere to human umbilical vascular endothelial cells more than CD16– cells,\(^{24}\) thereby giving rise to a potential mechanism for monocyte migration into the failing myocardium. Furthermore, CD16\(^+\) monocytes have higher proinflammatory activity than CD16– cells, and in vivo studies have demonstrated higher cytokine production by monocytes (including IL-1\(\beta\), IL-8, and MCP-1) in response to MPA formation.\(^{20–25}\) Consequently, MPA formation is also associated with promoting a greater inflammatory phenotype in monocytes.\(^{24,28}\)

Phagocytosis is one of the most important functions of monocytes, and studies have shown that this activity is particularly marked in Mon1 and Mon2 monocytes.\(^{11}\) The increased formation of MPAs in these subsets (in both AHF and SHF) might also reflect a mechanism of eliminating activated platelets from the circulation by phagocytosis. It is, therefore, possible that the high number of MPAs in HF may reflect the increased number of activated platelets found in this condition. In addition to the direct formation of MPAs, monocytes and platelets may also interact with each other to augment individual intrinsic function. For example, tissue factor release by monocytes in HF interacts with P-selectin on the surface of platelets, which results in enhanced fibrin formation.\(^{23,29}\)

Table 3. Monocytes, Platelets, and MPAs in Acute Heart Failure on Admission, Discharge, and at 3 Months of Follow-Up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Admission</th>
<th>Discharge</th>
<th>Follow-Up</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes, per µL</td>
<td>893 (336)</td>
<td>853 (255)</td>
<td>858 (331)</td>
<td>0.73</td>
</tr>
<tr>
<td>Platelets, per µL</td>
<td>249 (62)</td>
<td>271 (105)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>201 (59)</td>
<td>0.011</td>
</tr>
<tr>
<td>Total MPA, per µL</td>
<td>133 (99–180)</td>
<td>133 (96–172)</td>
<td>124 (95–197)</td>
<td>0.43</td>
</tr>
<tr>
<td>MPA with Mon1, per µL</td>
<td>105 (79–153)</td>
<td>106 (73–150)</td>
<td>94 (68–150)</td>
<td>0.40</td>
</tr>
<tr>
<td>MPA with Mon2, per µL</td>
<td>16 (10–24)</td>
<td>13 (7.5–19)</td>
<td>16 (6.6–29)</td>
<td>0.51</td>
</tr>
<tr>
<td>MPA with Mon3, per µL</td>
<td>9 (7–15)</td>
<td>11 (8–15)</td>
<td>10 (6–18)</td>
<td>0.61</td>
</tr>
<tr>
<td>Mon1 with MPA, %</td>
<td>17 (12–23)</td>
<td>17 (13–23)</td>
<td>14 (11–20)</td>
<td>0.33</td>
</tr>
<tr>
<td>Mon2 with MPA, %</td>
<td>24 (15–29)</td>
<td>22 (17–27)</td>
<td>19 (16–27)</td>
<td>0.78</td>
</tr>
<tr>
<td>Mon3 with MPA, %</td>
<td>15 (13–19)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>15 (11–18)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>13 (8.4–17)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Normally distributed data are presented as mean (SD), nonnormally distributed data are presented as median (interquartile range). MPA indicates monocyte-platelet aggregate; Mon1, CD14+CD16– monocytes; Mon2, CD14++CD16+CCR2+ monocytes; and Mon3, CD14+CD16++CCR2– monocytes.

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<td>0.61</td>
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<tr>
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<td>0.33</td>
</tr>
<tr>
<td>Mon2 with MPA, %</td>
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<td>22 (17–27)</td>
<td>19 (16–27)</td>
<td>0.78</td>
</tr>
<tr>
<td>Mon3 with MPA, %</td>
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<td>15 (11–18)&lt;sup&gt;‡&lt;/sup&gt;</td>
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</table>
Patients with HF have been shown to have increased plasma MCP-1, a key monocyte chemoattractant. In this study there was a significant correlation between the plasma levels of MCP-1 and counts of MPA associated with nonclassic Mon3. This is perhaps in accordance with previous evidence showing the intimate role that platelets play in the regulation of monocyte recruitment to tissues. In addition, these observations once more indicate very complex and context-dependent patterns of monocyte-platelet interaction. For example, the P-selectin--mediated pathway has been shown to be pivotal for monocyte aggregation with platelets in the settings of atherothrombosis but not in endotoxemia.

The results of our study show that increased MPA formation in Mon1 and Mon2 monocytes after acute decompensation of HF persists for ≥3 months, despite improvements in patient symptoms. Our findings suggest that the inflammatory mechanisms that activate monocytes and platelets continue many weeks after the initial event, which may contribute to the considerable morbidity and mortality seen in this patient group. Therapeutic modulation of thrombotic substrates in HF is an attractive concept, particularly in patients with AHF in whom inflammation and thrombosis are greatest. Reductions in MPAs have been demonstrated after administration of monoclonal antibodies against P-selectin glycoprotein ligand 1 and the blockade of P-selectin, and although antiplatelet therapy seems to reduce MPAs, anticoagulation has little effect. At present, there are no convincing clinical trial data to suggest a beneficial role on mortality of either anticoagulation or...
antiplatelet therapy in patients with HF, unless there are concomitant indications, such as CAD or atrial fibrillation.

Limitations

The numbers of study subjects is relatively small. Although we specifically recruited HF patients with underlying CAD to draw comparisons with an appropriate control group, residual confounding may remain with some differences in clinical characteristics present between the study groups. Also, our findings should be evaluated in patients with HF because of other aetiologies, such as dilated cardiomyopathy. The functional implications of the observed changes will require further study, because it remains unclear whether MPA formation is simply a reflection of the underlying thromboinflammatory state in HF or whether they directly contribute to the pathophysiology of the disease process. Larger studies are also required to explore the use of MPAs as a risk stratifying marker for clinical outcomes in HF and to investigate the effects of using established and novel antithrombotic/anti-inflammatory drugs on both monocyte and platelet activation in this condition.

Conclusions

For the first time, we have shown that MPA formation is increased in patients with HF, and this may provide some preliminary evidence of an interaction between inflammation and thrombosis in HF. In AHF, the increase in MPAs seems to be confined to monocytes from Mon1 and Mon2 subsets, which have been shown in other studies to be proinflammatory and highly phagocytic. Also, MPAs with Mon2 seem to be negatively predictive of a worse prognosis in AHF, perhaps reflecting a beneficial role for these complexes during acute decompensation. However, larger studies powered for clinical outcomes are required to confirm whether MPAs have any prognostic role in patients with HF.

Sources of Funding

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Disclosures

None.

References

Monocytes play important roles in cardiovascular disease, and their actions can be both beneficial (eg, angiogenesis or cardiovascular repair) or detrimental (eg, excessive inflammatory response). This diversity is partly attributable to the existence of different monocyte subpopulations, and mechanisms regulating their individual mobilization, recruitment to tissues, and functional status in cardiovascular disease are still poorly understood. Recent findings have indicated a pivotal role of monocyte-platelet interactions in the coordination of cellular activity, and the present study shows for the first time significant upregulation of monocyte-platelet aggregates in patients with acute and chronic heart failure. Moreover, changes in the number of such aggregates, proportions of monocytes involved, and associations with plasma levels of monocyte chemoattractant protein 1 are specific to individual monocyte subsets. Specifically, in acute heart failure, increased monocyte-platelet aggregates were confined to monocytes from Mon1 subset (which have been shown in other studies to be proinflammatory and highly phagocytic) and Mon2 subset (which display a reparative phenotype). Furthermore, counts of aggregates with Mon2 were negatively predictive of a worse prognosis in acute heart failure, perhaps reflecting a beneficial role for these complexes after acute decompensation. In the future, therapeutic modulation of monocyte interactions with platelets may become a new target for the management of heart failure.
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Supplemental Table 1. Correlation analysis between monocyte platelet aggregates and plasma interleukin-6 and monocyte chemoattractant protein-1 in patients with acute heart failure

<table>
<thead>
<tr>
<th></th>
<th>Interleukin-6</th>
<th>Monocyte-chemoattractant protein-1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Total MPA, per µl</td>
<td>-0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>MPA with Mon1, per µl</td>
<td>0.01</td>
<td>0.92</td>
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<td>MPA with Mon2, per µl</td>
<td>-0.12</td>
<td>0.40</td>
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<tr>
<td>MPA with Mon3, per µl</td>
<td>0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>Mon1 with MPA, %</td>
<td>-0.03</td>
<td>0.86</td>
</tr>
<tr>
<td>Mon2 with MPA, %</td>
<td>-0.02</td>
<td>0.91</td>
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<td>Mon3 with MPA, %</td>
<td>0.03</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Mon1: CD14+CD16- monocytes, Mon2: CD14++CD16+CCR2+ monocytes, Mon3: CD14+CD16++CCR2- monocytes, MPA: monocyte-platelet aggregates
**Supplemental Table 2.** Correlation analysis between monocyte platelet aggregates and left ventricular ejection fraction and brain natriuretic peptide in patients with acute heart failure

<table>
<thead>
<tr>
<th></th>
<th>Left ventricular ejection fraction</th>
<th>Brain natriuretic peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Total MPA, per µl</td>
<td>-0.26</td>
<td>0.087</td>
</tr>
<tr>
<td>MPA with Mon1, per µl</td>
<td>-0.27</td>
<td>0.068</td>
</tr>
<tr>
<td>MPA with Mon2, per µl</td>
<td>-0.06</td>
<td>0.70</td>
</tr>
<tr>
<td>MPA with Mon3, per µl</td>
<td>-0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>Mon1 with MPA, %</td>
<td>-0.30</td>
<td>0.046</td>
</tr>
<tr>
<td>Mon2 with MPA, %</td>
<td>-0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>Mon3 with MPA, %</td>
<td>-0.12</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Mon1: CD14+CD16- monocytes, Mon2: CD14++CD16+CCR2+ monocytes, Mon3: CD14+CD16++CCR2- monocytes, MPA: monocyte-platelet aggregates