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Original article

Small-size Microparticles as Indicators of Acute Decompensated State in Ischemic Heart Failure

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ABSTRACT

Introduction and objectives: Microparticles are markers for cell activation and apoptosis and could provide valuable information that is not available from clinical data. This study assesses the clinical and biological relationship of small-sized microparticles in different forms of ischemic systolic heart failure and their relation to markers of inflammation and repair.

Methods: We compared 49 patients with acute heart failure, 39 with stable heart failure and 25 patients with stable coronary artery disease. Small-size microparticles counts were determined by high-resolution flow cytometry. Moreover, 3 different monocyte subpopulations and their expression of inflammatory and adhesive scavenger receptors were analyzed using a conventional flow cytometer.

Results: Endothelial CD144+ microparticle counts were decreased in heart failure groups ($P = .008$). Annexin V-binding microparticle counts were found increased in heart failure ($P = .024$) and in patients with lower functional class ($P = .013$). Platelet CD42b+ microparticle counts positively correlated with left ventricular ejection fraction ($P = .006$), and annexin V-binding microparticle counts with interleukin-6 levels in stable heart failure ($P = .034$). Annexin V-binding microparticle counts in the acute status strongly correlated with toll-like receptor-4 expression on all monocyte subsets (all $P < .01$). Three months after admission with acute heart failure, annexin V-binding microparticle counts were positively correlated with receptors for interleukin-6, CD163 and CD204 (all $P < .05$).

Conclusions: Annexin V-binding microparticle counts constitute valuable hallmarks of acute decompensated state in systolic heart failure. The observed relationship between small-size annexin V-binding microparticles and scavenger receptors supports their involvement in the progression of the acute response to injury, and thus their contribution to the pathogenesis of acute decompensated heart failure.

Micropartículas de pequeño tamaño como indicadores del estado agudo en la insuficiencia cardiaca sistólica

Palabras clave:
Micropartículas de pequeño tamaño
Insuficiencia cardiaca
Monocito
Receptores barredores

RESUMEN

Introducción y objetivos: Las micropartículas son marcadores de la activación celular y la apoptosis y podrían aportar una información muy valiosa e inasequible con los datos clínicos. En este estudio se evalúa la relación clínica y biológica entre las micropartículas de pequeño tamaño presentes en diferentes formas de la insuficiencia cardiaca sistólica isquémica y los marcadores de la inflamación y la reparación.

Métodos: Se compararon 49 pacientes con insuficiencia cardiaca aguda, 39 con insuficiencia cardiaca estable y 25 pacientes con enfermedad coronaria estable. Se cuantificaron las micropartículas de pequeño tamaño mediante citometría de flujo de alta resolución. Se analizaron también tres subpoblaciones monocitarias diferentes y su expresión de receptores barredores de la inflamación y la adhesión empleando un citómetro de flujo convencional.

Resultados: El recuento de micropartículas CD144+ de origen endotelial mostró reducción en los grupos con insuficiencia cardiaca (p = 0,008). Se observó que el recuento de micropartículas unidas a anexina V aumentaban en la insuficiencia cardiaca (p = 0,024) y en los pacientes con peor clase funcional (p = 0,013). El recuento de micropartículas CD42b+ de origen plaquetario presentaron una correlación positiva con la fracción de eyección del ventrículo izquierdo (p = 0,006), y los de micropartículas unidas a anexina V presentaron correlación positiva con la concentración de interleucina 6 en la insuficiencia cardiaca estable (p = 0,034). En el estado agudo, el recuento de micropartículas unidas a anexina V mostró intensa correlación con la expresión del receptor *toll-like-4* en todos los subgrupos de monocitos

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(p < 0,01 en todos los casos). Tres meses después del ingreso por insuficiencia cardiaca aguda, el recuento de micropartículas unidas a anexina V tenía correlación positiva con los receptores de interleucina 6, CD163 y CD204 (p < 0,05 en todos los casos).
Conclusiones: El recuento de micropartículas unidas a anexina V es una valiosa característica distintiva del estado agudo descompensado en la insuficiencia cardiaca sistólica. La relación observada entre las micropartículas de pequeño tamaño unidas a anexina V y los receptores barredores respalda su intervención en la progresión de la respuesta aguda a la lesión y, por lo tanto, su contribución en la patogenia de la insuficiencia cardiaca aguda descompensada.

Abbreviations

AHF: acute heart failure
CAD: coronary artery disease
sAMP: small-size annexin V-binding microparticles
sEMP: small-size endothelial microparticles
SHF: stable heart failure
sPMP: small-size platelet microparticles

INTRODUCTION

Systolic heart failure (HF) remains a debilitating and life-threatening condition despite considerable treatment achievements.[1,2] Many major pathways for prothrombotic and proatherogenic changes and endothelial damage converge on an ischemic or hypertensive heart and the molecular processes represent a complex network of interacting pathways.[3]

Microparticles are valuable markers for cell activation and apoptosis.[4] A previous study reported that endothelial microparticles levels reliably predict future cardiovascular events in patients with HF, suggesting that microparticles could be involved in the pathogenesis of several cardiovascular conditions apart from atherothrombotic complications.[5] In addition, circulating microparticles are increased in subjects with cardiovascular risk factors and coronary artery disease (CAD),[6,7] being recently related to indices of injury and repair in patients with acute coronary syndrome and independent predictors of future HF in non—ST-segment elevation myocardial infarction patients.[8] Besides, the role of monocytes in ischemic conditions is uncertain. These functions include inflammatory response, regulation of thrombogenic status (eg, via tissue factor expression and modulation of fibrinolysis), but also beneficial properties related to scavenging of redundant/dangerous substances, angiogenesis, and repair.[9,10] In view of the interrelationships between vascular dysfunction, inflammation, and apoptosis with cardiac function, it is conceivable that microparticles could potentially be a diagnostic marker related to pathophysiology of acute heart disease.[11]

In the present study, our objective was to investigate the relevance of microparticles in HF, using an approach that allows discrimination and quantification of a wide range of microparticles sized 0.1 μm polystyrene beads or above (eg, "small-size" microparticles).[12] This size range corresponds to the accepted definition of microparticles.[13] We hypothesized that small-size microparticles generation and origin are related to distinct pathomechanisms assessed as monocytic activation indexes, and ultimately to the damage and severity of the disease. We therefore examined the relationship of circulating counts of small-size microparticles in patients with acute HF (AHF) and stable HF (SHF) of ischemic origin, who were compared to "disease control" patients with stable CAD and preserved left ventricular function. Additionally, we investigated relationships of small-size microparticles counts to expression of scavenger receptors on monocytes as markers of inflammation and repair to provide insight about the pathophysiological status of the disease.

METHODS

Study Population

In the following prospective study, we consecutively recruited 49 patients with AHF and 39 patients with SHF. The AHF group (all in New York Heart Association [NYHA] functional class IV) 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.[14] Patients with SHF were recruited from outpatient clinics (NYHA I-III); they presented with chronic HF with no deterioration in clinical condition. All patients with HF had left ventricular ejection fraction (LVEF) of ≤ 40% on echocardiography or left ventriculography. In order to evaluate the impact of HF, only patients with underlying CAD as the etiology of HF were recruited. Patients with acute coronary syndrome were excluded (chest pain with ST/T wave changes on electrocardiogram ± positive troponin). Elapsed time between the last acute episode and hospitalization was > 6 months.

This would allow comparisons to be made with a suitable control group with CAD (n = 25), preserved ventricular function, and no HF, but with a similar pattern of comorbidities, risk factors (eg, diabetes, hypertension) and background medication. "Disease controls" with stable CAD were defined as myocardial infarction > 6 months previously and/or angiographically documented stenosis > 50% in ≥ 1 coronary artery and LVEF ≥ 55 %. For all study groups, exclusion criteria included factors that could affect small-size microparticles counts and monocyte phenotype (infectious and inflammatory disorders, cancer, creatinine > 200 μmol/L, steroids and hormone replacement therapy), atrial fibrillation, or moderate-severe valvular disease.

Blood Collection and Time-points

For laboratory analysis, non-fasting peripheral venous blood samples were collected from all participants and processed by flow cytometry within 60 minutes for assessment of monocyte characteristics (fresh whole blood). Platelet-poor plasma was frozen and stored for consequent batched small-size microparticles and cytokine analysis (details below).

In order to assess the dynamics of small-size microparticles and monocyte parameters in AHF over time, blood samples were analyzed at the following time-points: *a)* during the first 24 hours after admission; *b)* on the day of hospital discharge, and *c)*