High correlation between 2 flow cytometry platforms in the microparticles analysis using a new calibrated beads strategy

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Microparticles (MPs) are potential noninvasive biomarkers for diagnosis or prognosis in pathologic conditions. However, the lack of standardization of the preanalytical and analytical methods leads to a wide variability in MPs results. The recently developed Megamix-Plus beads, a new bead-based standardization tool optimized to specific types of flow cytometers, could help circumvent this problem. The aim of the present study was to determine whether the number of total MPs and platelet-derived MPs (PMPs) is similar using 2 different cytometer platforms calibrated with the Megamix-Plus beads. Blood samples from 65 patients with deep venous thrombosis were collected and processed to obtain platelet poor plasma (PPP). The number of total MPs and PMPs in each PPP sample was measured using 2 flow cytometers. Megamix-Plus side scatter channel beads were used to calibrate the LSRFortessa flow cytometer from Becton Dickinson, whereas Megamix-Plus forward scatter channel beads were applied to the Navios flow cytometer from Beckman Coulter. High correlation of total MPs and PMPs values between the flow cytometers was found ($r = 0.908$, $P < 0.01$ and $r = 0.910$, $P < 0.001$, respectively). However, the absolute numbers of total MPs and PMPs were significantly higher measured with the Navios flow cytometer compared with the LSRFortessa cytometer. Therefore, both platforms are valid for MPs determination in general, although a similar platform with the same calibration tool could be a better choice for multicenter studies. (Translational Research 2015;166:733–739)

Abbreviations: MPs = microparticles; PPP = platelet poor plasma; PMPs = platelet MPs; BC = Beckman Coulter; BD = Becton Dickinson; FS/FSC = forward Scatter/channel; SS/SSC = side Scatter/channel; PE = phycoerythrin; PECy7 = phycoerythrin-Cyanine7; FITC = fluorescein isothiocyanate; FL1 = fluorescence 1 channel

INTRODUCTION

Microparticles (MPs) are microvesicles from 0.1 to 1 μm in diameter, which are released from different types of cells during cell activation or apoptotic processes.[1,2] They present cell-specific antigens and cytoplasmic markers and generally express phosphatidylserine on their surface.[3,4] MPs were initially considered to be "cellular dust" without any

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AT A GLANCE COMMENTARY

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Background

Microparticles (MPs) have emerged as promising noninvasive biomarkers for various diseases. Flow cytometry is the most common method to detect MPs, but it is a technical challenge because of the small size of MPs leading to a wide variability in MPs results. A new bead-based standardization tool optimized to specific types of flow cytometers could help circumvent this problem.

Translational Significance

We observed high correlations in total MPs and platelet-derived MPs between 2 different cytometers in spite of optimal comparison of absolute MPs numbers remains to be achieved. These results are relevant for further multicenter studies.

biological function. They are now, however, of clinical relevance because of their role as vectors and biomarkers for diagnosis or prognosis in vascular damage, blood coagulation, inflammation, angiogenesis, and other pathologic situations.[4-9] Nonetheless, a lack of standardization of the preanalytical and analytical methods has led to a wide variability in the number of MPs detected.[10-14] Flow cytometry is the most common method to detect MPs, which allows the determination of both the number and the cellular origin of MPs based on the antigens presented on their surface. Reliable measurement of MPs by flow cytometry is a technical challenge because of the small size of MPs, which is close to the limit of sensitivity for flow cytometers.[15] New sets of fluorescent beads have recently been developed. Megamix-Plus forward scatter channel (FSC) beads (Biocytex, Marseille, France) are designed to obtain the best results using flow cytometers with optimized FSC, such as Beckman Coulter (BC) flow cytometers, whereas Megamix-Plus side scatter channel (SSC) beads (Biocytex, Marseille, France) are more adequate for flow cytometers with optimized SSC, such as Becton Dickinson (BD) flow cytometers. To compare the results between 2 different cytometry platforms (BD and BC flow cytometers), we measured the number of total MPs and platelet-derived MPs (PMPs) in patients with deep venous thrombosis using Megamix-Plus calibration beads.

PATIENTS AND METHODS

Blood collection and sample processing. Sixty-five patients diagnosed with deep venous thrombosis were included in the study. The research was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written consent was obtained from all subjects according to a protocol approved by the Ethical Committee of the Virgen del Rocio Hospital. Venous blood was taken with a 21-gauge needle and with minimal compression and collected (discarding the first 3 mL) in 0.109-M sodium citrate Vacutainer plastic tubes (BD, Erembodegem, Belgium). Blood samples were transported in a vertical position to laboratory and processed within 2 hours of blood extraction. Platelet poor plasma (PPP) was obtained by centrifugation at 1500 g for 30 minutes at 4°C. Collection of PPP was stopped 1 cm above the buffy coat to avoid cell contamination. The PPP was stored as aliquots at −80°C until analysis. All blood samples were processed at the Virgen del Rocio Hospital, and aliquots of PPP samples were shipped to La Fe Hospital under optimal freezing conditions.

MPs and PMPs characterization by flow cytometry. The concentrations of total MPs and PMPs in PPP from each patient were measured in both, a BD LSRFortessa flow cytometer in the Virgen del Rocio Hospital and a BC Navios flow cytometer in the La Fe Hospital. The reagents and protocol for sample labeling in the 2 hospitals were strictly the same. Thirty microliters of frozen PPP, which had been frozen for a month, were thawed at room temperature[11,14,16] and incubated with monoclonal antibodies (mouse antihuman CD31-phycoerythrin, mouse antihuman CD41-phycoerythrin–cyanine7; Beckman Coulter, Marseille, France) and with Annexin V–fluorescein isothiocyanate (FITC; Trevigen, Gaithersburg, MD) that binds to phosphatidylserine exposed on the MPs surface in 500 μL of HEPES dilution buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$; pH, 7.4) for 1 hour on ice in the dark. To limit background noise from dust and crystals, all reagents were double filtered with 0.22-μm filters.

Before MPs detection, each cytometer was first calibrated with their corresponding Megamix-Plus fluorescent beads. The setting of the BC flow cytometer (Navios; Beckman Coulter, Brea, CA) was fixed using Megamix-Plus FSC fluorescent polystyrene beads (0.1, 0.3, 0.5, and 0.9 μm; Biocytex, Marseille, France) according to the manufacturer's instructions. Megamix-Plus SSC fluorescent polystyrene beads (0.16, 0.20, 0.24, and 0.5 μm; Biocytex, Marseille, France) were used to calibrate the BD flow cytometer (LSRFortessa Becton Dickinson, Erembodegem, Belgium). In both instruments, all cytometer parameters were selected at a logarithmic scale. Although the ranges of the sets of beads are different, these beads are designed to establish