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Cell-derived microvesicles in infective endocarditis: Role in diagnosis and potential for risk stratification at hospital admission

Milton Henriques Guimarães Júnior[a], Teresa Cristina Abreu Ferrari[a], Andréa Teixeira-Carvalho[b], Marcela de Lima Moreira[b], Lorena Júnia de Souza Santos[b], Matheus Fernandes Costa-Silva[b], Rodrigo Matos Pinto Coelho[a], Pedro Henrique Oliveira Murta Pinto[a], Tijmen Hermen Ris[c], Jonathas Teixeira Salles[a], Lívia Silva Araújo Passos[a], Maria Carmo Pereira Nunes[a,*]

[a] *Programa de Pós-Graduação em Infectologia e Medicina Tropical e Departamento de Clínica Médica, Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[b] *Fundação Oswaldo Cruz, Instituto René-Rachou - FIOCRUZ Minas, Laboratório de Biomarcadores de Diagnóstico e Monitoração, Belo Horizonte, Brazil*
[c] *Erasmus University Rotterdam - Erasmus MC, Netherlands*

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SUMMARY

Objectives: To characterize the plasmatic profile of cell-derived microvesicles (MVs) at diagnosis and during the treatment of patients with infective endocarditis (IE).
Methods: Blood samples from 57 patients with IE were obtained on 3 consecutive moments: upon admission (T0), at 2 weeks (T1), and at the end of treatment (T2), and were compared with 22 patients with other bacterial infections. MPs were measured by flow cytometry and labeled for specific cell markers of CD45 (leukocytes), CD66b (neutrophils), CD14 (monocytes), CD41a (platelets), CD51 (endothelial cells), CD3 (T lymphocyte) and CD235a (erythrocytes).
Results: MVs from platelets (pltMVs), leukocytes (leukMVs), neutrophils (neutMVs), monocytes (monoMVs) and lymphocytes (lymphMVs) were significantly more elevated in the patients with IE, compared to the patients with other bacterial infections, despite comparable age, sex, blood counts and C-reactive protein levels. MVs values revealed a relatively stable pattern over time in IE, except for a significant increase in leukMVs and neutMVs in T1. LeukMVs ($p = 0.011$), neutMVs ($p = 0.010$), monoMVs ($p = 0.016$) and lymphMVs ($p = 0.020$), measured at admission, were significantly higher in IE patients that died during hospitalization in comparison with those that survived. In a multivariable analyses, the levels of neutMVs remained as an independent factor associated with mortality (odds ratio 2.203; 95% confidence interval 1.217 - 3.988; $p = 0.009$), adjustment for heart failure during the treatment.
Conclusions: Plasma levels of pltMVs, leukMVs, neutMVs, monoMVs and lymphMVs were significantly more elevated in patients with IE than in patients with other bacterial infections at hospital admission. Furthermore, neutMVs at admission have been identified as an independent predictor of mortality in patients with IE. Thus, cell derived MPs may become an important tool in the differential diagnosis and mortality risk assessment early in the course of IE suspected cases.

Introduction

Infective endocarditis (IE) is the microorganism infection of the endocardium known for its high morbidity and mortality in spite of treatment.[1] The clinical presentation and outcome are largely determined by the immune and inflammatory response influenced by the host-pathogen interaction. The complex pathogenesis of this condition is a challenge, and understanding the immune-inflammatory cell pathways can be a step closer to improve disease management.

In the last decades, studies have demonstrated a role of cell-derived microvesicles (MVs) in the immune-inflammatory response in several diseases. MVs are small vesicles, typically around 100–1000 nm in size, released from the plasma membrane by activated, injured, or apoptotic cells.[2,3] Although there is no consensual definition of MVs,[4] MVs are larger than exosomes, their density is unknown and they are usually isolated at 10,000 to 20,000 x g by

* Corresponding author.
E-mail address: mcarmo@waymail.com.br (M.C.P. Nunes).

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centrifugation. MVs are often called microparticles (MPs), although the term "microparticles" has also been used for total populations of vesicles isolated from human plasma at 100,000 x g and such populations will contain exosomes.[5]

The release of MVs have been demonstrated in physiological[6] and pathological conditions acting as intercellular messengers.[7] The number and constitution of the released MVs depends on the cell type and its state, and on the environmental conditions.[8] MVs have been studied in sepsis,[9–12] where they were demonstrated to be associated with microvascular dysfunction, organ damage[9] and coagulation abnormalities.[13] MVs have also been associated with thromboembolic events in patients with cancer,[14] and with plaque instability in coronary artery disease.[15] Some studies demonstrated that the total number of MVs, including the MVs derived from platelets, monocytes and endothelium, was significantly higher in patients with type 2 diabetes mellitus than in non-diabetic controls.[16,17] In chronic obstructive lung disease, endothelium derived MVs have been associated with the grade of lung destruction, airflow limitation and disease exacerbation.[18]

Taking all together, it is possible that MVs participate in the pathophysiology of IE. However, up to this date, there are no studies evaluating the profile of plasmatic MVs in IE. Therefore, the aim of our study was to characterize the profile of MVs released by different cells in IE, compare their kinetics during the disease treatment, and evaluate their potential for predicting clinical outcome.

Methods

Between August 2011 and January 2017, 65 patients with definite IE, according to the Dukeś Modified Criteria,[19] consecutively admitted to the University Hospital, Federal University of Minas Gerais, Brazil were evaluated for inclusion in the study. Exclusion criteria were IE patients who were taking antibiotics for more than one week prior to the moment of inclusion in the study, and those who died or underwent cardiac surgery before the collection of the first blood sample for MVs measurement. The Institutional Ethics Committee approved the study protocol and written informed consent was obtained from all the participants.

After being included in the study, the patients were followed during hospitalization, and their clinical, laboratorial and echocardiographic data were recorded in the study protocol. Blood samples for MVs measurement were obtained at 3 consecutive times, as follows:

T0	At the diagnosis of infective endocarditis
T1	At the 15th day of antibiotic treatment
T2	Before hospital discharge

Blood sample for MVs quantification was collected before the beginning of the antibiotics or within the first week of treatment, we meant that the T0 sample was collected at the time of diagnosis, preferably before the beginning of the antibiotics. Patients who had already been taking antibiotics at the time of diagnosis had the T0 sample collected only if they had been taking it for less than 7 days, otherwise they were excluded from the study. At the later situation, it was not collected at the same day for every single patient because the time from antibiotic initiation and the definition of the IE diagnosis varied among them. Then, T1 samples were collected at day 15th of antibiotic treatment for every single patient, and T2 after completing the treatment, before hospital discharge.

The endpoint analyzed in this study was overall mortality related to any complication of IE during hospitalization.

The data of the patients with IE were compared with those of a control group, consisting of 22 patients with other bacterial infections that presented with fever and elevated C-reactive protein (CRP) serum levels. The other infections comprised pyelonephritis (8 cases), pneumonia (7 cases), catheter-related bloodstream infection (6 cases), and monoarthritis (1 case) without any organ dysfunction and any other sign of severity. Samples from the control group were collected only once, i.e. by the time of their enrollment in the study. As for the IE group, blood sample for MVs quantification was collected before the beginning of the antibiotics or within the first week of the use of these agents.

Sample preparation and MVs measurement

Citrated peripheral blood samples (3.2 mL) was centrifuged at 3000 × g for 15 min, and then the plasma was cooled at −20 °C before storage at −80 °C. Immediately before analyses, the samples were thawed at 37 °C and were further centrifuged at 13,000 × g for 3 min to obtain platelet-free plasma. The latter was diluted 1:3 in citrated phosphate buffered saline (PBS) containing heparin and centrifuged at 14,000 × g for 90 min at 15 °C. The resultant MVs pellet was then resuspended in 1 × annexin V binding buffer (BD Biosciences, CA).

MVs were measured by flow cytometry. MVs selection was based on particle size, presence of a common surface marker (phosphatidylserine) and specific surface antigens according to the cell origin as described elsewhere.[20–22] In the first step, the MVs isolated from plasma were gated (R1) based on their forward (FSC) and side (SSC) scatter distribution compared to the distribution of synthetic 0.7–0.9 µm SPHERO™ Amino Fluorescent Particles (Spherotech Inc. Libertyville, IL). After that, events present in R1 were accessed for their positive staining for annexin V (BD Bioscience, CA), which binds to phosphatidylserine. To properly place gates, mouse IgG PE conjugated isotype control monoclonal antibodies (mAbs) were used. Finally, annexin *V*+ events were gated with conjugated mAbs against the cell markers CD45-PE (leukocytes), CD66b-PE (neutrophil), CD14-PercCP (monocytes), CD3-PE (T lymphocyte) CD41a-PercCP (platelets), CD235a-PECy5 (erythrocytes) and CD51/61-PE (endothelial cells).

All reagents and mAbs used in the flow cytometry experiments were from BD Biosciences (Becton-Dickinson, CA), unless otherwise stated. The samples were analysed in a FACScalibur flow cytometer (Becton-Dickinson, CA). Over 100,000 events were acquired on each sample to reach at least 2000 events within the MVs gate.

The cytometer was set to operate at a high flow rate setting for 60 s for each sample. The number of MVs/µL of plasma was calculated as described elsewhere[23]: $MVs/\mu L = (N \times 400)/(60 \times 100)$, in which N is the number of events, 400 is the total volume of the tube before analysis, 60 is the sample volume analyzed, and 100 is the original volume of MPs suspension used to perform the phenotyping protocol.

Normal reference values

As normal reference values we used the ones obtained in the same research laboratory (Instituto René Rachou, FIOCRUZ Minas), with similar protocol analyses, derived from 30 healthy blood donors. MVs reference values were: until 75 counts/µL for leukocytes MVs (leukMVs); until 75 counts/µL for neutrophils MVs (neutMVs); until 75 counts/µL for monocytes MVs (monoMVs); until 45 counts/µL for T lymphocytes MVs (lymphMVs); until 90 counts/µL for platelets MVs (pltMVs); until 45 counts/µL for erythrocytes MVs (eryMVs); and until 54 counts/µL for endothelium MVs (endoMVs).

Statistical analysis

Categorical variables, expressed as numbers and percentages, were compared using chi-square testing, whereas continuous data, expressed as median and interquartile range (IQR), were compared

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Table 1
Baseline characteristics of the study population.

Variable	Value
Age (years)	50 (39–64)
Male	33 (58.0)
Diabetes mellitus	9 (15.8)
Chronic renal disease	13 (22.8)
Predisposing conditions	
Rheumatic valve disease	17 (29.8)
Degenerative valve disease	9 (15.7)
Mitral valve prolapse	11 (19.2)
Congenital heart disease	5 (8.7)
Central venous catheter	7 (12.0)
Previous infective endocarditis	2 (3.4)
Clinical findings	
Fever	45 (80.0)
Weightloss	30 (53.0)
Anorexia	37 (65.0)
Musculoskeletal manifestation	12 (21.0)
Heart murmur	44 (77.0)
Microbiologic etiology	
Coagulase-negative *Staphylococcus*	15 (26.3)
Streptococcusspp	7 (12.3)
Staphylococcus aureus	6 (10.5)
Gram negative rods	5 (8.8)
Enterococcus spp	3 (5.3)
Fungi	2 (2.5)
Negative culturefindings	17 (29.8)
Complications and outcomes	
In-hospital death	17 (30.0)
Early surgery	25 (44.0)
Development of heart failure	29 (51.0)
Fever >10 days (on treatment)	8 (14.0)
Neurologic event	11 (19.0)

Data are expressed as absolute numbers (percentage) and median (interquartile range).

Table 2
Demographical and laboratorial features of the patients with IE compared with the patients with other bacterial infections.

Variable	Cases ($n=57$)	Controls ($n=22$)	P value
Age (years)	51.0 (39–64)	56.6 (23–67)	0.411
Male (n/%)	33 (58)	10 (41)	0.457
C-reactive protein (mg/L)	76 (37–183)	139 (50–240)	0.610
Hemoglobin (g/dl, mean)	10 (9–11)	11.5 (9–13)	0.007
Leukocytosis (x10^3/µl)	11 (7–14)	10 (5–13)	0.218
Platelets (x10^3/µl)	181 (137–251)	186 (115–267)	0.732
Creatinin (mg/dl)	1.08 (0.8/1.7)	0.97 (0.8/1.2)	1.000
Microvesicles (counts/µL)			
Leukocytes	560 (239–832)	313 (200–503)	0.032
Neutrophils	275 (138–478)	135 (94–265)	0.023
Monocytes	202 (121–428)	110 (82–210)	0.051
Lymphocytes	200 (130–303)	119 (58–241)	0.034
Erythrocytes	166 (85–305)	111 (58–217)	0.110
Endothelium	428 (244–728)	242 (212–380)	0.054
Platelets	536 (341–1119)	317 (218–492)	0.020

Data are expressed as the median (interquartile range).

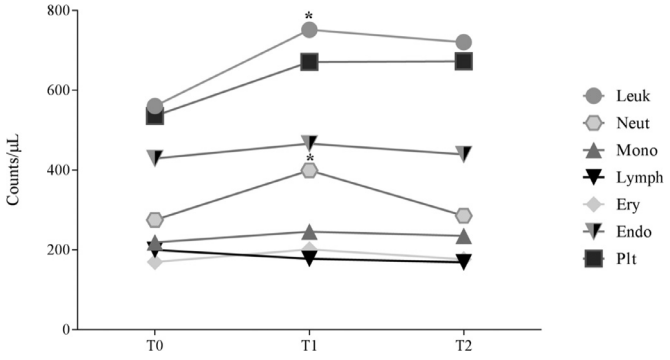

Fig. 1. MVs levels from different phenotypes, at T0 (admission), T1 (two weeks of treatment) and T2 (end of treatment) in IE patients. Data are expressed as median. Leuk = microvesicles from leukocytes; Neut = microvesicles from neutrophils; Mono = microvesicles from monocytes; Lymph = microvesicles from T lymphocytes; Ery = microvesicles from erytrocites; Endo = microvesicles from endothelium; Plt = microvesicles from platelets. Statistically significant differences ($p < 0.05$) between the times T0 and T1 are indicated by an asterisk above the symbol related to each group.

using the Mann-Whitney U test, as appropriate. Logistic regression model was constructed to test the independent association between MVs concentrations and death, after adjustment for other covariates. The data were analyzed using SPSS 23.0 statistical software (SPSS, Chicago, IL) and Prism GraphPad software, version 5 (San Diego, CA).

Results

The baseline characteristics of the 57 patients classified as having definitive IE are shown in Table 1. Median age of the IE patients was 50 years (IQR 39–64 years), and 33 (58%) individuals were male. Native valve IE was observed in 32 (56%) cases, followed by prosthetic valve IE in 15 patients (26%) and device-related IE in 10 (17.5%). The most common predisposing condition was rheumatic valve disease (29.8%) and the most common comorbidity was chronic renal disease (22.8%). One patient had HIV infection, none was illicit drug user. The most prevalent agent was coagulase-negative *Staphylococcus,* which was isolated in 15 (26%) of the cases, followed by *Streptococcus* spp (12%) and *S. aureus* (10%). Culture-negative IE represented 29.8% of the cases.

Transesophageal echocardiography was performed in 47 (82%) of the patients and transthoracic echocardiogram in the remaining 10 (17.5%). Vegetations were identified in 49 patients (86%) and 20 (35%) vegetations were described as >10 mm.

There was no significant difference between the IE group and the group with other bacterial infections regarding age, CRP levels, hemoglobin concentrations, white blood cell counts, platelets counts and creatinin levels (Table 2).

A high proportion (>84%) of patients in the IE group and in the control group of the other bacterial infections presented levels of all MVs phenotypes above the reference values. The comparison of the MV counts between the group of other bacterial infections and IE patients (at admission) demonstrated significantly higher levels of the leukMVs ($p=0.032$), neutMVs ($p=0.023$), lymphMVs ($p=0.034$) and pltMVs ($p=0.020$) in the IE patients

Microvesicle counts during treatment

During hospital stay, 17 patients died with an overall mortality rate of 30%. Twenty-five patients (44%) were submitted to cardiac surgery, chiefly due to development of heart failure (51%). The median time between the diagnosis and the surgery was 9 days (IQR 3–17 days). Neurologic events occurred in 11 (19.3%) patients and the most prevalent was ischemic stroke, which was observed in 9 (15.8%) individuals.

Fig. 1 shows MVs levels during treatment course. LeukMVs, pltMVs and endoMVs presented the highest levels. There were a higher count of leukMVs ($p=0.049$) and neutMVs ($p=0.033$) in T0 compared with T1.

To analyze the potential value of MVs in predicting early mortality, initially we compared MV levels at admission (T0) and at T1 between the patients who died within the first 2 weeks and those who survived at least 2 weeks after diagnosis. We found that the counts of leukMVs ($p=0.018$), neutMVs ($p=0.009$), monoMVs ($p=0.015$) and lymphMVs ($p=0.019$) measured at T0 were significantly higher in the patients who died compared to those who

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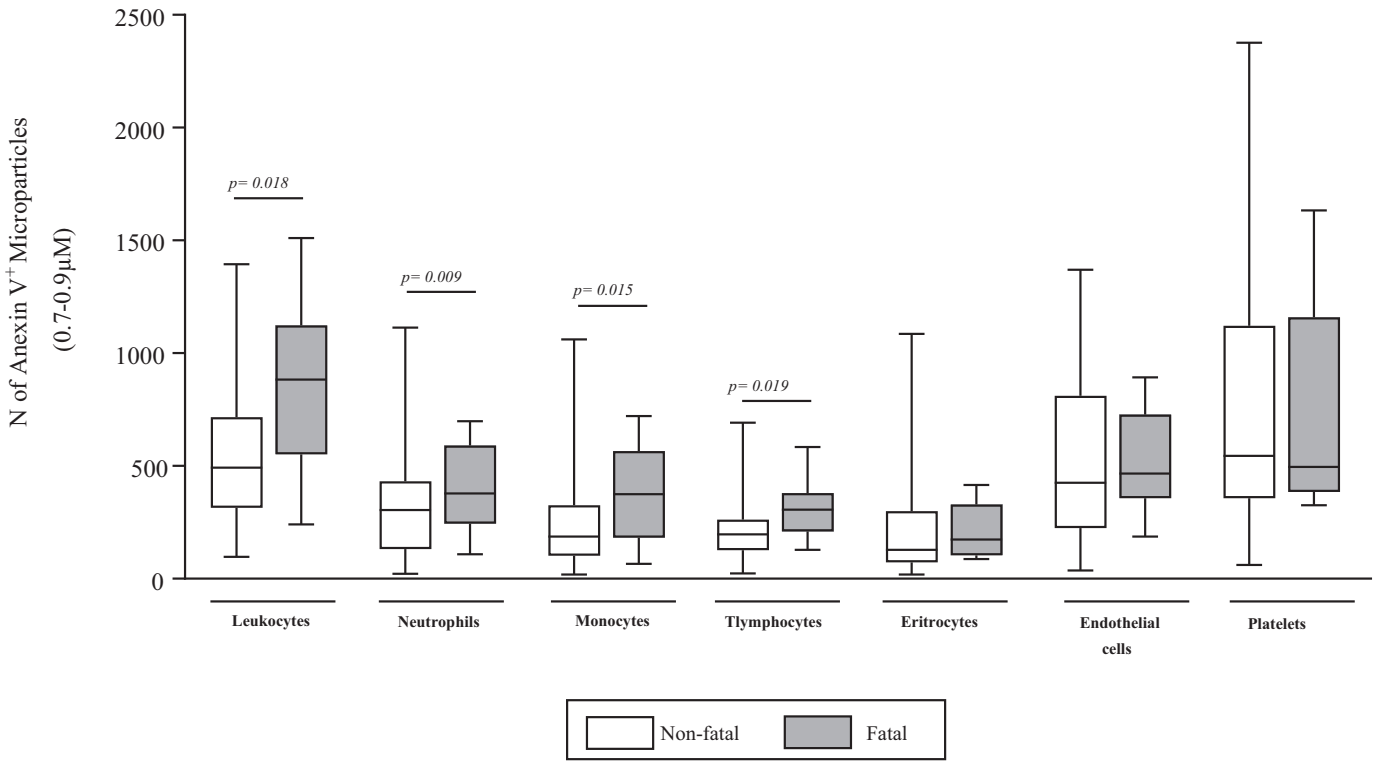

Fig. 2. Comparison between levels of microvesicles (MVs) at T0 (admission), in infective endocarditis patients who survival (white bars) or died (grey bars) during hospitalization. The results are expressed as percentage in box plot format. The box extends from the 25th percentile to 75th percentile, with a horizontal line at the median (50th percentile). Whiskers extend from the lowest value to the 25th percentile and from the 75th percentile to the highest value, showing the range of data distribution. Statistical significance is indicated in each graph.

survived (Fig. 2). MV levels at different times (T0, T1, T2) are shown in Fig. 3 (A and B). Although pltMVs levels at T1 were higher in those who died, this difference was not significant, as there were a wide variation in pltMVs during the disease evolution. Additionally, mean platelet volume (MPV) values, which were 8.7 ± 2.6fL presented no differences between the patients who died and survived.

There were no significant differences in MV levels at admission concerning the need of surgery, cerebral event, or development of heart failure. In the logistic regression analyses, the levels of neutMVs at admission remained as an independent predictor of death, after adjustment for worsening of heart failure during the course of IE (odds ratio 2.203; 95% CI 1.217–3.988; $p = 0.009$).

Discussion

As far as we know, this is the first study describing the plasma concentrations and kinetics of MVs in patients with IE, and also comparing IE MV levels with those observed in other bacterial infections. All MVs phenotypes demonstrated elevated plasma levels during infection (IE and the other bacterial infections groups) when compared with reference values defined in a healthy population. Despite comparable age, sex, white blood cell count and CRP levels, we observed significantly higher baseline levels of leukMVs, neutMVs, lymphMVs and pltMVs in the IE group. This suggests a greater activation of these cells in IE resulting in higher MVs production. MVs levels stratified by mortality showed higher levels of leukMVs, neutMVs, monoMVs and lymphMVs at admission in the patients who died compared with those who survived. After adjustment for development of heart failure, which is the main complication of IE, neutMVs at baseline remained predictor of death.

Extracellular vesicles (EVs) encompass a broad range of vesicles released from cells.[3] EVs can be classified into different subsets according to their size, cellular origin, content or the mechanism leading to their formation. At present, at least 3 main subgroups of EVs have been defined. MVs, also referred to as microparticles, are vesicles typically around 100–1000 nm in size.[3,24,25] Exosomes are smaller cell-derived vesicles that are present in all biological fluids. Their diameter is between 30 and 100 nm, the density ranges between 1.13 and 1.19 g/ml and the morphology has been described as cup-shaped. Apoptotic bodies are larger vesicles containing nuclear materials. In our study we have analyzed EVs from 500 nm to 900 nm, thus our population was constituted of MVs, without overlap with exosomes.

In the IE patients, the highest levels of MVs were derived from leukocytes, followed by those derived from platelets and endothelial cells. Studies that evaluated patients with sepsis[26] and meningococcal disease[27] demonstrated similar findings. The elevation of neutMVs levels found in our patients with IE is also similar to the results of Timár et al.[28] that demonstrated a 6-fold increase in the release of neutMVs, comparing patients with *S. aureus* bacteremia with healthy individuals.

In some studies concerning other diseases in which MVs counts were performed, the authors observed a predominant increase in pltMVs differently from what was observed in the present study, in which leukMV counts were very similar to those of pltMVs in both cases and controls with other infections. As those studies included patients who were critically-ill, including meningococcal infection with septic shock,[27] acute *Plasmodium vivax* malaria,[20] and sepsis admitted to an intensive care unit,[26] and probably presented an acute phase response more exacerbated than that exhibited by our cases, the intensity of the acute phase response and the more acute character of the infection may be possible explanations for the observed difference. However, the possibility of influence by pre-analytical factors cannot be ruled out.

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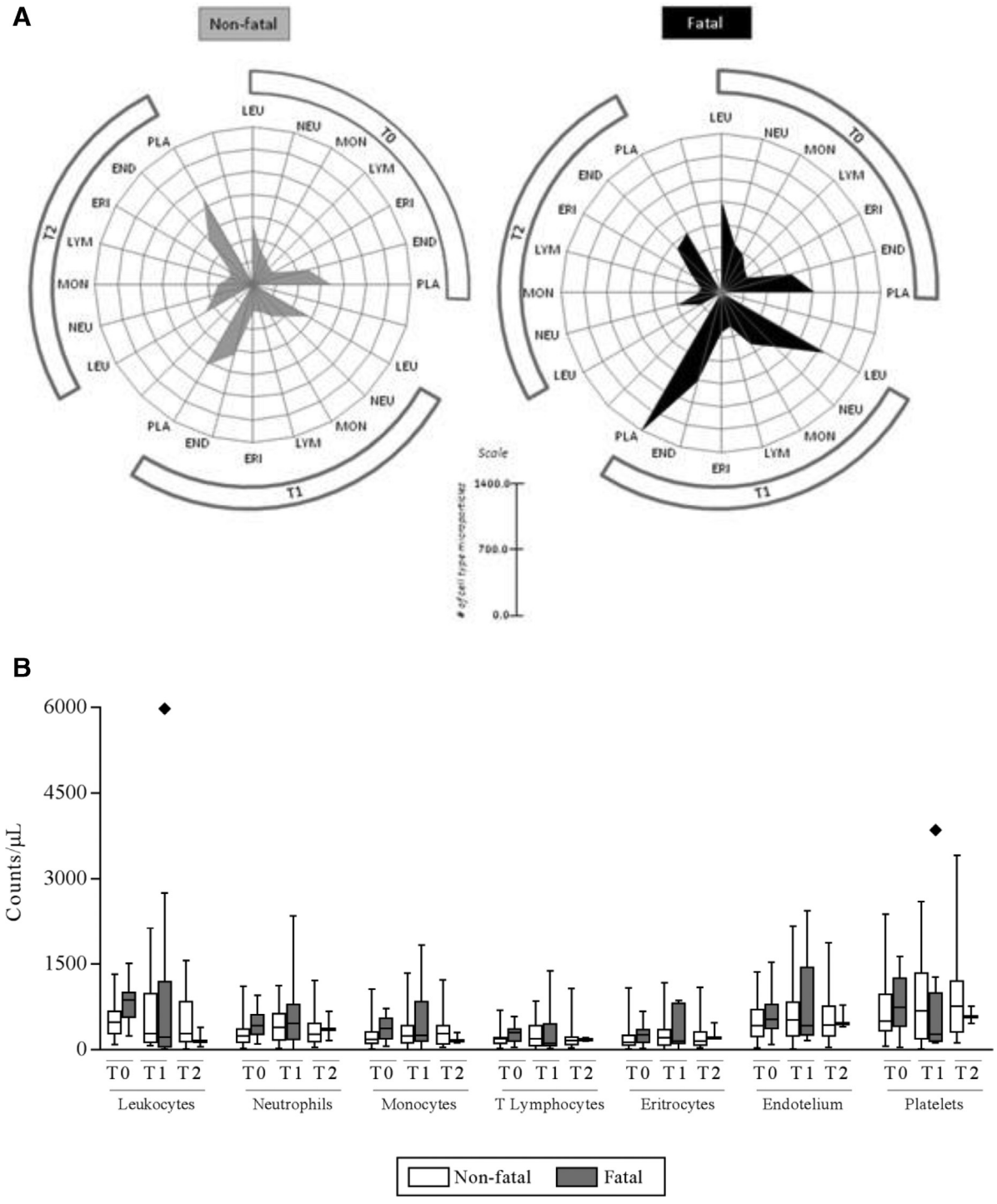

Fig. 3. A. Radar graphics showing MVs levels according to the cell origin at different times (T0, T1, T2) in survivals and non-survival patients. T0 = admission; T1 = 2 weeks of treatment; T3 = end of treatment; Leu = MVs from leukocytes; Neu = MVs from neutrophils; Mon = MVs from monocytes; Lym= MVs from T lymphocytes; Eri = MVs from erytrocites; End = MVs from endothelium; Pla = MVs from platelets.
3B Kinetics of circulating microvesicles (MVs) from endocarditis patients, according to non-fatal and fatal cases at different times (T0, T1, T2). T0 = admission; T1 = 2 weeks of treatment; T2 = end of treatment. The results are expressed as percentage in box plot format. The box extends from the 25th percentile to 75th percentile, with a horizontal line at the median (50th percentile). Whiskers extend from the lowest value to the 25th percentile and from the 75th percentile to the highest value, showing the range of data distribution. Outliers values was plotted as individual points.

Microvesicles kinetics during the treatment

There are few studies reporting MVs kinetics, determined by flow cytometry, during treatment of infectious diseases; furthermore, most of them are cross-sectional and none of them in IE. We observed increase in the leukMVs and neutMVs serum concentrations from admission (T0) to the end of the second week of treatment in the IE patients (T1). An interesting observation was the course of pltMVs in surviving patients. Overtime, the pltMVs tended to increase which may represent a protective role of pltMVs during IE. This in agreement with a study that found a negative correlation between pltMVs and organ dysfunction in the

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patients who died due to severe sepsis.[29] However, on T1, nonsurviving group had an important increase in pltMVs as well, but the pltMV levels were lowered again at T2.

It can be speculated a possible influence of cardiac surgery on this finding as in our cohort, 44% of the patients underwent surgery, in a median of 9 days after the diagnosis (before obtaining data of T1). However, the half-life of MVs seems to be very short. Rank et al.[30] reported that pltMVs half-life in humans is 5.8 h.[30] A shorter time (90% clearance after 30 min) was reported in an animal study of exosomos released by erythrocytes.[31] Furthermore, Fu et al.[32] did not find any difference in the total MVs concentrations comparing the MP levels before surgery with those obtained 12 and 72 h after the procedure. A possible explanation for this finding is the above described short half-life of MVs. Thus, we cannot attribute the enhancement of leukMV and neutMV levels observed in our study to the surgical procedure. Nevertheless, it is known that cardiopulmonary bypass surgery provokes an inflammatory state that is able to induce MVs release.[33]

Value of microparticles in predicting mortality

The mechanisms that stimulate MVs release are cell activation and apoptosis, in a process involving cytokine and endotoxin release, complement lysis, oxidative stress and high shear stress,[34,35] which may predict disease severity.

The association between MVs levels and clinical outcomes has been investigated in other diseases. Zafrani et al.[36] demonstrated that preventing the release of MVs was associated with a decrease in microvasculature dysfunction and higher survival in animal models of sepsis. Delabranche et al.[13] found increased leukMVs levels in patients with sepsis that developed disseminated intravascular coagulation. In agreement with our finding that neutMVs were predictors of mortality during hospitalization, a previous study showed a high mortality rate in septic mice injected with neutMVs.[37]

Other authors have explored the mechanisms that could explain the association between leukMVs, neutMVs, monoMVs or lymphMvs and high mortality. When looking for mechanisms, is important to take into account that the role of MVs depends on their composition, which is related to the cell of origin and the target cell. MVs from T lymphocytes are capable of inducing pro-inflammatory peptides, nitric oxide-synthesis and cyclooxygenase-2 expressions, especially in the middle layer of the vessels.[38] MonoMVs induce, in a concentration-dependent manner, reactive oxygen species production, cytokine release and nuclear factor kappa B (NF-Kb) activation in monocytes and macrophages.[39]

Illustrating the complexity of MVs effects, neutMVs have been reported to be pro or antiinflamatory, depending on the target cell.[40] Gasser et al.[41] observed that neutMVs are capable of provoking a dose-dependent release of the anti-inflammatory cytokine transforming growth factor $\beta 1$ (TGF-$\beta 1$) on macrophages, as well as a decrease in the release of interleukin (IL)-6, CXCL-8 and tumor necrosis factor alpha (TNF-α). Johnson et al.[37] demonstrated in animal model that the administration of neutMVs was associated with increase in bacterial load and IL-10 levels, and a decrease in macrophage activation. Otherwise, it was demonstrated that neutMVs caused a pro-inflammatory response on platelets[42] and endothelial cells.[43] As an effect of the IE antimicrobial therapy, the ongoing bacteria destruction and clearance cause continuing antigen exposure, increasing the stress and pro-inflammatory factors release, resulting in activation of neutrophils and elevation of neutMV levels during the course of treatment, especially during the initial phase when the destruction of bacteria is more intense. However, further research, comparing the MVs levels with the release of cytokines, endotoxins, complement lysis, oxidative stress and high shear stress could clarify their effects. Irrespective of their role, plasmatic counts of MVs, specifically neutMVs, may be a useful prognostic tool for risk assessment during the hospitalization of IE patients. The possible mechanisms implicated in this poor outcome, as well as the final MVs effect (by enhancing a pro or an antiinflamatory state) deservers more investigation.

In this context, knowledge about the role of platelets in the development and severity of various conditions, in addition to thrombosis, continues to appear, especially in relation to the areas of inflammation and immune response.[44] As elevated mean platelet volume (MPV) indicates increased platelet activation, this index has been studied in several disorders. Some authors observed that the MPV was higher in patients with IE than in health controls and decreased significantly after treatment.[45,46] It has also been demonstrated that IE patients who present embolic events had increased MPV values, compared to those who do not present this complication.[47,48] Furthermore, previous history of IE, *S. aureus infection*, end-stage renal disease, depressed left ventricular ejection fraction, early surgical intervention, vegetation size $\geq$10 mm, presence of perivalvular abscess, higher on-admission platelet count, CRP and MPV levels emerged as independent predictors of in-hospital poor outcomes.[48] Thus, high MPV values may be a biomarker of unfavorable outcome in IE, especially regarding the increased risk of thromboembolic events. Additionally, it is possible to speculate that platelets may play a role in the mortality of these patients. However, it was demonstrated that several diseases, including local or systemic infections and many drugs may potentially affect MPV levels.[48] Thus, further studies taking into account the factors that may affect MPV are necessary to assess the actual associations between MPV and IE.

The lower limit of detection of the cytometer that was used in our investigation (0.7 µm) did not allow the detection of smaller MVs, which is a limitation of the present study. In addition, our sample size and number of deaths limited the possibility to adjust for more variables in the multivariable analyses.

Conclusions

This study demonstrated that MVs derived from platelets, leukocytes, neutrophils, monocytes and lymphocytes were significantly more elevated in patients with IE than in patients with other bacterial infections. MVs concentration from leukocytes and neutrophils were higher at IE diagnosis than at 2 weeks of treatment. Moreover, MVs from neutrophils have been identified as an independent predictor of mortality in patients with IE. Therefore, cell derived MVs could become an important tool in the differential diagnosis and mortality risk assessment of patients with suspected IE at admission. However, studies with larger sample size are needed before drawing definitive conclusions.

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