Circulating microparticles from septic shock patients exert differential tissue expression of enzymes related to inflammation and oxidative stress

Maria Letizia Mastronardi, PhD; Hadj Ahmed Mostefai, PhD; Ferhat Meziani, MD, PhD; Maria Carmen Martínez, PhD; Pierre Asfar, MD, PhD; Ramaroson Andriantsitohaina, PhD.

INSERM, U771, Angers, France; CNRS UMR, 6214, Angers, France; Université d'Angers, Angers, France (MLM, HAM, FM, MCM, RA); Laboratoire HIFIH, UPRES EA 3859, IFR 132, Université d'Angers, PRES UNAM (PA); Département de Réanimation Médicale et de Médecine Hyperbare, Centre Hospitalo- Universitaire, Angers, France (FM, PA)

Present address (FM): Service de réanimation médicale, Nouvel Hôpital Civil, Hôpitaux universitaires de Strasbourg, Strasbourg, France; Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, Strasbourg France

Correspondence to Dr. Ramaroson Andriantsitohaina, INSERM, U771, Faculté de Médecine, Rue Haute de Reculée, Angers, F-49045 France. e-mail: ramaroson.andriantsitohaina@univ-angers.fr

Supported by institutional grants from Fondation pour la Recherche Médicale (n° INE20050303433), Fonds Européen pour le Développement Régional (n° 8891). R.A. is supported by a Contrat d'Interface Inserm. H.A.M. is recipient of a doctoral fellowship from Conseil Régional du Pays de la Loire.

Short running title: Tissue effects of septic microparticles

Keywords: Microparticles; sepsis; shock; nitric oxide; oxidative stress; inflammation.

Abstract

Objective: Septic shock is characterized for hypotension and multiple organ failures after infection by microorganisms. Septic patients exhibit high levels of circulating microparticles, small vesicles released from plasma membrane of activated or apoptotic cells. Here, we have investigated the effects of in vivo injection of microparticles from nonseptic or septic subjects on protein expression in mice tissues.

Design: Prospective, controlled experiments.

Setting: Animal basic science laboratory.

Subjects: Male Swiss mice were assigned randomly to one of two groups: 11 animals injected with microparticles isolated from healthy subjects, and 15 animals injected with microparticles isolated from septic patients.

Interventions: Microparticles were extracted from whole blood of septic and nonseptic subjects and were *i.v.* injected in mice. After 24 hours, mice were sacrificed and heart, lungs, liver and kidneys were isolated for western blots assays. Also, organs were used for direct measure of nitric oxide (NO) and superoxide anion (O_2^-) productions by electron paramagnetic resonance.

Measurements and Main Results: In heart and lungs, microparticles from septic shock patients increased expressions of endothelial and inducible NO-synthases, cyclo-oxygenase-2 and NF-κB. Only extracellular superoxide dismutase (SOD) expression was increased in heart. These effects were associated either with a greater oxidative or nitrative stress in heart and lungs, respectively, without affecting NO production. Liver exhibited an increase in oxidative stress linked to decreased endothelial NO-synthase and Mn-SOD expressions. However, cyclo-oxygenase 2 expression and IκBα phosphorylation were decreased. Septic microparticles did not change O_2^- and NO productions in kidneys.

Conclusions: Altogether, these data suggest that microparticles from septic shock patients exert pleiotropic and differential effects depending on targets tissues with regard to the expression of pro-inflammatory proteins related with nitrative and oxidative stresses and they might participate in organ dysfunction observed in septic shock patients.

INTRODUCTION

Sepsis, the host response to infection, may lead to severe sepsis that is associated with evidence of organ dysfunction as a consequence of the combination of severe inflammation and secondary changes in endocrine profile. During this phase, the cardiovascular system adopts a high cardiac output state with low peripheral resistance hemodynamic profile, and hypotension that is refractory to catecholamines despite adequate volume resuscitation, and contributes to life-threatening organ failure during septic shock (1, 2). Release of reactive oxygen species (ROS) by different pathways contributes to the failure of organ such as lung, heart, liver and kidney. Indeed, there is a considerable body of evidence for redox imbalance and oxidative stress in sepsis demonstrated by increased markers of oxidative damage (for review see 3, 4).

Besides, the activation of a number of host mediator systems, including the cytokine, leukocyte, and hemostatic networks may lead to overproduction of nitric oxide (NO). Large amounts of NO, generated by enhanced expression of the inducible NO synthase (iNOS) have been detected in human vessels from septic patients (5) and from endotoxinic rats (6), and are implicated in symptoms observed in septic shock patients and animals, including tissue hypoperfusion and hypoxia, lactic acidosis, oliguria and vascular hyporeactivity.

Recently, it has been shown that microparticles (MPs) derived from the plasma of malaria-infected mice induce potent activation of macrophages in vitro and may contribute to malaria infection-induced inflammation (7). Also, Soriano et al. (8) have shown that MPs could be considered as markers of inflammation in patients with septic shock. Moreover, Niewland et al (9) have reported elevated levels of circulating MPs in meningococcal sepsis. MPs are small vesicles shed from the blebbing plasma membrane of various cell types during activation or apoptosis (10). Recently, we have shown that circulating MPs are significantly increased in septic patients compared to nonseptic subjects and in particular, platelet- and

endothelial-derived MPs, as well as, L-selectin$^+$ and P-selectin$^+$ MPs (11). Furthermore, MPs from septic patients are rather protective against vascular hyporeactivity in order to maintain vasoconstrictive response in mice treated with LPS (11). To the best of our knowledge, the role played by MPs in different target organs has not been assessed although they regulate vascular function in septic shock patients. Moreover, their role with regard to tissular changes either of oxidative or nitrative stress during sepsis is not known. Therefore, the aim of the present study is to assess the *in vivo* pathophysiological relevance of MPs after they were *i.v.* injected into mice, with respect to NO and O_2^- productions in heart, liver, lungs and kidneys.

Materials and methods

Patients and blood cell preparation

This study was approved by the Ethics committee of the Société de Réanimation de Langue Française. The study included patients with (n = 15) or without (n = 11) septic shock. Septic shock was defined according to standard criteria of American college of chest physicians/Society of Critical Care Medicine Consensus Conference, 1992. Baseline characteristic of patients with septic shock (male: 93%, female: 7%) are shown in Table 1. Also, the sources of sepsis are shown in Table 1. Patients without evidence of infection and with normal hemodynamics were considered as controls (male: 73%, female: 27%).

Peripheral blood (20 mL) from nonseptic and septic patients was collected during the early phase of septic shock (10 ± 4 hours after enrollment in the intensive care unit) from a peripheral vein. MPs were isolated and MPs subpopulations were discriminated according the expression of membrane-specific antigens and samples were analyzed in a MXP (Beckman Coulter) as previously reported (11). The MPs concentration for injection was determined by flow cytometry. As previously described (11), septic patients displayed elevated circulating levels of MPs from platelets and endothelial cells, and those expressing CD62L and CD62P. Levels of endotoxin were assessed in all MP preparations with a Limulus amebocyte lysate kit (QCL-1000; Lonza, Walkersville, MD) and were found to be below the lower detection limit of the kit (<0.1 endotoxin unit/mL).

NO determination by electron paramagnetic resonance (EPR)

All animal studies were carried out using approved institutional protocols and were conformed the *Guide for the Care and Use of Laboratory Animals* published by US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Swiss mice (8-10 weeks old) were treated *in vivo* by injection into the tail vein of MPs at the circulating levels of MPs detected in the blood of septic or nonseptic patients (range: 1,451 – 69,485 MPs/microliter of

plasma), as previously described (11). It should be noted that independently of the circulating level, all septic MPs displayed hyperreactivity in aortic rings (11). Under these conditions, we did not see any mortality or clinical signs of sepsis in injected mice.

Hearts, livers, lungs and kidneys were obtained from mice treated either with MPs from nonseptic or septic patients during 24 hours. Then, tissues were dissected and incubated for 30 minutes in Krebs-Hepes buffer containing: BSA (20.5 g/L), $CaCl_2$ (3 mM) and L-arginine (0.8 mM) in order to assess NO production. $Fe(DETC)_2$ solution was added to organs and incubated for 45 minutes at 37°C. Then, organs were immediately frozen using liquid N_2. NO measurement was performed on a table-top x-band spectrometer Miniscope (Magnettech, MS200, Berlin, Germany) as previously described (11). Values are expressed in unit/mg weight of dried tissue.

Superoxide anion spin-trapping

After sacrifice of mice pre-treated with nonseptic and septic MPs, heart, liver, lungs and kidneys were allowed to equilibrate in deferoxamine-chelated Krebs-Hepes solution containing 1-hydroxy-3methoxycarbonyl-2,2,5,5-tetramethylpyrrolidin (CMH, Noxygen, Germany) (500 µM), deferoxamine (25 µM) and DETC (5 µM) under constant temperature (37°C) for 45 minutes. Samples were then frozen in liquid N_2 and analyzed by EPR. Values are expressed in unit/mg weight of dried tissue.

Western Blotting

After treatment, tissues were homogenized and lysed. Proteins (80 µg) were separated on 10 % SDS-PAGE. Blots were probed with anti-endothelial NOS (eNOS), anti-inducible NOS (iNOS), anti-cyclooxygenase-2 (COX-2) (BD Biosciences, San Jose, CA), anti-cyclooxygenase-1 (COX-1) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-p65 subunit of nuclear factor-κB (NF-κB) (Abcam, Cambridge, UK), anti-phospho-IκB alpha (p-IκBα) (US Biological, Swampscott, MA), anti-Mn superoxide dismutase (Mn-SOD), anti-copper/zinc

superoxide dismutase (Cu/Zn-SOD), anti-extracellular superoxide dismutase (EC-SOD) (Stressgen Biotechnologies Corporation, Victoria, Canada) and anti-nitrotyrosine (Cayman Chemical, Ann Arbor, MI) antibodies. A polyclonal rabbit anti-β-actin antibody (Sigma-Aldrich, St Quentin Fallavier, France) was used for visualization of protein gel loading. The membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences, Piscataway, NJ), and the protein-antibody complexes were detected by Enhanced chemiluminescence plus (Amersham Biosciences).

Data analysis

Data are represented as mean ± SEM, *n* represents the number of animals. Statistical analyses were performed by two-way analysis of variance or nonparametric Mann-Whitney U tests depending on the number of samples. $P<0.05$ was considered to be statistically significant.

Results

Effects of MPs on protein expression in heart. Hearts taken from mice treated with septic MPs exhibited enhanced expressions of enzymes related to NO and prostanoid productions, namely eNOS and iNOS, COX-1 and COX-2, compared with that taken from nonseptic MP-treated mice. These effects were associated with an increase in NF-κB expression without modification of p-IκBα. Concerning the enzymes implicated in oxidative stress, treatment with septic MPs elicited a greater expression of EC-SOD but not Cu/Zn-SOD nor Mn-SOD (Fig. 1).

Effects of MPs on protein expression in lungs. In lungs from septic MP-treated mice (Fig. 2), eNOS expression was increased, as well as, expression of inducible proteins, iNOS and COX-2 but not that of COX-1. In addition, NF-κB expression was augmented whereas p-IκBα was significantly reduced. No effects on SOD protein expressions were observed in lungs treated with MPs from septic patients.

Effects of MPs on protein expression in liver. As shown in Fig. 3, livers from septic MP-treated mice exhibited lower expressions of eNOS and COX-2. Expressions of iNOS, COX-1 and NF-κB were not modified in livers from septic MP-treated mice compared with that from nonseptic-treated mice. Moreover, p-IκBα was significantly reduced. Although MPs from septic patients induced a slight, but significant, decrease in Mn-SOD expression, the other SOD proteins were not modified.

Effects of MPs on protein expression in kidneys. As shown in Fig. 4, kidneys from septic MP-treated mice exhibited a significant decrease of eNOS expression, while those of COX-1, iNOS and COX-2 were not modified. Among other proteins studied, only p-IκBα was reduced and Cu/Zn-SOD was increased in response to septic MP treatment.

Effects of MPs on tissue NO and O_2^- production and nitrative stress. NO measurement by EPR showed that septic MPs did not affect NO production, in heart, liver, lungs and

kidneys, compared with nonseptic MP-treated mice. Regarding oxidative stress, O_2^- production was greater in heart and liver but not in lungs and kidneys from mice treated with septic MPs versus nonseptic MPs (Fig. 5).

The reaction between NO and O_2^- generates peroxynitrite. Then, we evaluated its formation in tissues by studying nitration of proteins, a subsequent step to peroxynitrite formation, using specific antibodies raised against nitrotyrosine. Interestingly, septic MPs strongly increased nitration of proteins in lungs, but not in other tissues (heart, liver and kidneys) (Fig. 6).

Discussion

The present study show that i.v. injection of MPs obtained from septic shock patients, at an early septic stage, into mice exerts differential effects on target tissues of such as heart, liver, lungs, and kidneys with regard to proteins related to NO release and O_2^- production, as well as COX. Thus, heart and lungs were the most affected with respect to increased expression of pro-inflammatory proteins (iNOS, COX-2 and NF-κB pathway) but not those of SOD isoform except that of EC-SOD in the heart. These effects were associated either with a greater oxidative or nitrative stress in heart and lungs, respectively, without affecting tissular NO production. Liver exhibited an increase in oxidative stress linked with decreased eNOS and Mn-SOD despite reduced expression of COX-2 and phosphorylation of IκBα. Finally, kidneys were the least affected inasmuch septic MPs did not change O_2^- and NO productions.

Recently, we reported increased circulating levels of MPs in patients with septic shock compared to nonseptic patients (11). In the present study, we provide evidence that heterologous injection in mice of MPs obtained from septic shock patients compared to injection of MPs obtained from non-septic shock patients may induce deleterious effects in different target tissues including heart, lung and livers in terms of oxidative and nitrative stresses despite the fact that they may, rather, be protective in counteracting the drop in peripheral resistance and progressive hypotension during severe sepsis (11), under the experimental conditions used. Very recently, we found that rats with sepsis induced by peritonitis exhibit a specific phenotype of MPs, being MPs from leukocytes increased. Inoculation of these MPs in healthy rats reproduced hemodynamic, septic inflammatory patterns, associated with oxidative and nitrative stresses in heart and aorta (12). Although, septic MPs from human (11) or rats (12) exert different effect in terms of oxidative and nitrative stresses in aorta, they exhibit similar effects in the heart.

Whereas constitutive NOS appears to be involved in the physiological regulation of myocardial contractility through interaction between endothelial cells and cardiac myocytes (13), iNOS is responsible for the larger component of the prolonged myocardial depression during sepsis. Upon septic MP treatment, no increase in NO level was detected in the heart despite eNOS and iNOS over-expressions. In this tissue, it is possible that the strong increase in O_2^- production induced by septic MPs decreases the bioavailability of NO, as we previously described in metabolic syndrome (14). Also, uncoupling of NOS may explain the lack of detection of NO production. In fact, both eNOS and iNOS uncoupling can contribute to increase in O_2^- levels (15, 16). Thus in heart, enhanced expression of the two isoforms of NOS by septic MPs may account for the increase in O_2^- but not NO production. We have previously shown that injection of MPs isolated from rats with peritonitis increased both NO and O_2^- in MP-inoculated rats (12). These discrepancies suggest that the different origin of MPs (peritonitis in rats *vs* pulmonary infection mainly in septic patients) could account for the different regulation of NO production. Nevertheless, the cellular targets of septic MPs remained to be determined whether they originated from cardiomyocytes or inflammatory cells infiltrated in the myocardium.

In lungs, we can speculate that the scavenging of NO by O_2^- to produce peroxynitrite is a key mechanism that decreases NO bioavailability in lungs. This hypothesis is confirmed by the increase in nitration of proteins in lungs from mice treated with septic MPs. It was shown that several proteins (72, 42, 35, 31, 25, 9 Kda) were nitrated in lungs from mice treated with septic MPs. Although the identity of these proteins has not been assessed, their molecular weights were similar to those of COX-2, actin or ERK MAP kinase, MnSOD, caveolin and ubiquitin. Indeed, nitration of these proteins may have critical consequences in pathophysiology of the lung during sepsis.

In addition, in heart as well as in lungs, COX-2 expression was increased. Indeed, COX-2 is over-expressed in heart from septic rats and this is associated with heart injury (17). Also in septic shock rats, COX-2 metabolites are involved in the alterations of L-type calcium currents observed in myocytes (18). Marshall and collaborators (19) have shown an increased COX-2 levels in lungs from lipopolysaccharide-injected rats. Altogether, these results suggest that septic MPs may be responsible, at least in part, of the over-expression of COX-2 described in heart and lungs during sepsis and they may account for the deleterious effects of COX metabolites in these tissues.

The hepatic dysfunction and acute renal failure occur in the first hours after the initial injury as result of a decrease of portal and hepatic blood flows (20, 21). Although it is established that iNOS is induced in the liver and kidney during septic shock (22, 23), injection of septic MPs in mice neither affected NO production nor iNOS expression in liver, even both eNOS and COX-2 expressions were reduced. eNOS deficiency increases susceptibility to acute renal failure during sepsis (25). Thus, septic MPs may have a deleterious effect on hepatic and renal function by acting on endothelial level although they did not affect tissular production of NO.

Despite no changes or decrease on I-κBα phosphorylation has been observed in all tissues, NF-κB, iNOS and COX-2 expressions are increased in heart and lungs. It is possible that activation of I-κBα might be delayed compared to activation of downstream proteins or that upregulation of iNOS and COX-2 observed in tissues from mice treated with septic MPs involves transcription factor different to NF-κB.

Regarding the enzymes controlling oxidative stress, expression of EC-SOD was strongly increased in heart treated with septic MPs, and this was associated with O_2^- overproduction. EC-SOD is the only anti-oxidant enzyme that scavenges O_2^- specifically in the extracellular compartment. The fact that O_2^- levels increase concomitantly to the enhancement of EC-SOD suggests that the overproduction of O_2^- might induce an up-regulation of EC-SOD expression

in order to compensate the increase in oxidative stress. Other explanation is that the gain of EC-SOD function can be associated with deleterious effects as observed in other models. Indeed, the gain of SOD function is accompanied to motor neuron disease in transgenic mice (26) and atherosclerotic plaque development in ApoE-/- and LDL-/- mice (27). In the liver, septic MPs significantly increased O_2^- levels along with a slight reduction of Mn-SOD expression. Since Mn-SOD is mainly expressed in mitochondria, it is plausible to hypothesize that this organelle plays a role in the control of oxidative stress in liver. Septic MPs did not modify O_2^- production in the kidney despite an increase of Cu/Zn-SOD strengthened the notion that this organ is the least affected.

Limitations of the Study. We have reported that MPs may be protective against vascular hyporeactivity by maintaining a vasoconstrictive response in patients with septic shock (11). In the present study, we show, under the same experimental conditions, that septic MPs can induce oxidative and nitrative stresses and increase expression of pro-inflammatory enzymes in some of target tissues being heart and lungs the most affected. The reasons of the differential effects of MPs might be due to tissue distribution, metabolism or the nature of the target cells. In addition, it should be noted that the increase of nitration of proteins might imply not only an increase on peroxynitrite production, but also an enhanced activity of peroxidase enzymes, such as myeloperoxidases. Also, the effects of septic MPs on proteins implicated in coagulation have not been explored, although it is well established that MPs carry tissue factor and that in sepsis activation of coagulation has been described (28). Further studies are needed to sort out these underlying mechanisms. Nevertheless, we can conclude that MPs might participate in organ dysfunction observed in septic shock patients despite the reported correlation between increased circulating MPs and better survival rate among patients in the early phase of septic shock (8).

References:

1. Bone RC: Gram-positive organisms and sepsis. *Arch Intern Med* 1994;154:26-34.
2. Vincent JL, Korkut HA: Defining sepsis. *Clin Chest Med* 2008;29:585-590.
3. Andrades ME, Ritter C, Dal-Pizzol F: The role of free radicals in sepsis development. *Front Biosci* 2009;1:277-287.
4. Rinaldi S, Landucci F, De Gaudio AR: Antioxidant therapy in critically septic patients. *Curr Drug Targets* 2009;10:872-880.
5. Stoclet JC, Martinez MC, Ohlmann P, et al: Induction of nitric oxide synthase and dual effects of nitric oxide and cyclooxygenase products in regulation of arterial contraction in human septic shock. *Circulation* 1999;100:107–112.
6. Lanone S, Bloc S, Foresti R et al: Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implication for protection against endotoxic shock in rats. *FASEB J* 2005;19:1890-1892.
7. Couper KN, Barnes T, Hafalla JC, et al: Parasite-derived plasma microparticles contribute significantly to malaria infection-induced inflammation through potent macrophage stimulation. *PLoS Pathog* 2010;6:e1000744.
8. Soriano AO, Wenche J, Chirinos JA, et al: Levels of endothelial and platelet microparticles and their interactions with leukocytes negatively correlate with organ dysfunction and predict mortality in severe sepsis. *Crit Care Med* 2005;33:2540–2546.
9. Nieuwland R, Berckmans RJ, McGregor S, et al: Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* 2000;95:930-935.
10. Martínez MC, Tesse A, Zobairi F, et al: Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am J Physiol* 2005;288:H1004-H1009.
11. Mostefai HA, Meziani F, Mastronardi ML, et al: Circulating microparticles from patients with septic shock exert protective role in vascular function. *Am J Resp Crit Care Med* 2008;178:1148-1155.
12. Mortaza S, Martinez MC, Baron-Menguy C, et al: Detrimental hemodynamic and inflammatory effects of microparticles originating from septic rats. *Crit Care Med* 2009;37:2045-2050.
13. Shah AM, Anderson AH: Effects of endocardial damage on myocardial contraction. In: Myocardial response to acute injury. Parratt JR (Ed), Macmillan, Basic-stoke, 1992, pp. 153-169.
14. Agouni A, Lagrue-Lak-Hal AH, Ducluzeau PH, et al: Endothelial dysfunction caused by circulating microparticles from patients with metabolic syndrome. *Am J Pathol* 2008;173:1210-1219.
15. Carraway MS, Piantadosi CA, Jenkinson CP, et al: Differential expression of arginase and iNOS in the lung in sepsis. *Exp Lung Res* 1998;24:253-268.
16. Maarsingh H, Pera T, Meurs H: Arginase and pulmonary disease. *Naunyn Schmiedebergs Arc Pharmacol* 2008;378:171-184.
17. Nin N, Lorente JA, Fernandez-Segoviano P, et al: High-tidal volume ventilation aggravates sepsis-induced multiorgan dysfunction in a dexamethasone-inhibitable manner. *Shock* 2009;31:429-434.
18. Zhang XH, Li GR, Bourreau JP: The effect of adrenomedullin on the L-type calcium current in myocytes from septic shock rats: signaling pathway. *Am J Physiol Heart Circ Physiol* 2007;293:H2888-H2893.
19. Marshall M, Keeble J, Moore PK: Effect of a nitric oxide releasing derivative of paracetamol in a rat model of endotoxaemia. *Br J Pharmacol* 2006;149:516-522.

20. Asfar P, De Backer D, Meier-Hellmann A, et al: Clinical review: Influence of vasoactive and other therapies on intestinal and hepatic circulations in patients with septic shock. *Crit Care* 2004;8:170-179.
21. Simon F, Giudici R, Scheuerle A, et al: Comparison of cardiac, hepatic, and renal effects of arginine vasopressin and noradrenaline during porcine fecal peritonitis: a randomized controlled trial. *Crit Care* 2009;13:R113.
22. Knowles RG, Merrett M, Salter M, et al: Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. *Biochem J* 1990;270:833-836.
23. Schrier RW, Wang W: Acute renal failure and sepsis. *N Engl J Med* 2004;351:159-169.
24. Wang W, Mitra A, Poole B, et al: Endothelial nitric oxide synthase-deficient mice exhibit increased susceptibility to endotoxin-induced acute renal failure. *Am J Physiol* 2004;287:F1044-F1048.
25. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science* 1994;264:1772–1775.
26. Fukai T, Galis ZS, Meng XP, et al: Vascular expression of extracellular superoxide dismutase in atherosclerosis. *J Clin Invest* 1998;101:2101-2111.
27. Levi M, van der Poll T: Inflammation and coagulation. *Crit Care Med* 2010;38:S26-S34.

TABLE 1: Baseline characteristics of non-septic and septic patients.

	Non-septic patients (n=11)	Septic patients (n=15)
Age (years)	42 ± 17	63 ± 13
Sex ratio (M/F)	8/3	14/1
Mean infusion rate of norepinephrine	0	0.8 ± 0.8 µg/kg/min
Mortality at 28 days	0	33%
SAPS II	0	57 ± 19
SOFA score	0	10.8 ± 3.8
Body mass index (kg/m^2)	23 ± 3	28 ± 10
Mean arterial pressure (mmHg)		73.5 ± 4.6
Heart rate/min		95 ± 5
Source of infection		
Pulmonary		47%
Abdominal		27%
Soft tissue		20%
Urinary		0%
Unknown		6%
Type of infection		
gram-positive cocci		5
gram-positive bacillus		1
gram-negative bacillus		4
Not identified		5
Treatments before admission		
Beta-blockers	0/11	5/15
ACE inhibitor	0/11	2/15
Calcium blockers	0/11	2/15
Diuretics	0/11	3/15
Statins	0/11	3/15
Antithrombotic/antiaggregrant	0/11	7/15
Proton pump inhibitor	2/11	4/15

Antibiotics	0/11	3/15
Anti-depressants	0/11	5/15
Treatments during hospitalization		
Benzodiazepines		13/15
Morphine		9/15
Anesthetics		4/15
Curare		7/15
Norepinephrine/epinephrine		11/15
Antibiotics		15/15
Activated C protein		6/15
Insulin		12/15
Hydrocortisone		13/15

Definition of abbreviations: ACE = Angiotensin-converting enzyme; SAPS = simplified acute physiology score; SOFA = sequential organ failure assessment.

Legend of figures

Figure 1. Effects of microparticles from nonseptic (NonSMPs) or septic (SMPs) subjects on protein expression in heart. Mice were injected with MPs for 24 h and then, they were sacrificed and heart isolated for analysis of protein expressions. Blots were probed with anti-eNOS, anti-iNOS, anti-COX-1, anti-COX-2, anti-NF-κB, anti-p-IκBα, anti-Cu/Zn-SOD, anti-Mn-SOD or anti-EC-SOD antibodies. Data are representative of nine separate blots, and the densitometry values are expressed in arbitrary units (AU) as mean ± S.E.M. ** $P < 0.01$, *** $P < 0.001$ *vs.* NonSMPs.

Figure 2. Effects of microparticles from nonseptic (NonSMPs) or septic (SMPs) subjects on protein expression in lungs. Mice were injected with MPs for 24 h and then, they were sacrificed and lungs isolated for analysis of protein expressions. Blots were probed with anti-eNOS, anti-iNOS, anti-COX-1, anti-COX-2, anti-NF-κB, anti-p-IκBα, anti-Cu/Zn-SOD, anti-Mn-SOD or anti-EC-SOD antibodies. Data are representative of nine separate blots, and the densitometry values are expressed in arbitrary units (AU) as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs.* NonSMPs.

Figure 3. Effects of microparticles from nonseptic (NonSMPs) or septic (SMPs) subjects on protein expression in liver. Mice were injected with MPs for 24 h and then, they were sacrificed and liver isolated for analysis of protein expressions. Blots were probed with anti-eNOS, anti-iNOS, anti-COX-1, anti-COX-2, anti-NF-κB, anti-p-IκBα, anti-Cu/Zn-SOD, anti-Mn-SOD or anti-EC-SOD antibodies. Data are representative of nine separate blots, and the densitometry values are expressed in arbitrary units (AU) as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$ *vs.* NonSMPs.

Figure 4. Effects of microparticles from nonseptic (NonSMPs) or septic (SMPs) subjects on protein expression in kidneys. Mice were injected with MPs for 24 h and then, they were sacrificed and kidneys isolated for analysis of protein expressions. Blots were probed with anti-eNOS, anti-iNOS, anti-COX-1, anti-COX-2, anti-NF-κB, anti-p-IκBα, anti-Cu/Zn-SOD, anti-Mn-SOD or anti-EC-SOD antibodies. Data are representative of nine separate blots, and the densitometry values are expressed in arbitrary units (AU) as mean ± S.E.M. ** $P < 0.01$, *** $P < 0.01$ *vs.* NonSMPs.

Figure 5. Effects of microparticles on tissue NO and O_2^- production. Quantification of the amplitude of NO-Fe(DETC)$_2$ (A) O_2^--CMH (B) signals in mouse tissues treated with either nonseptic (NonSMPs) or septic (SMPs) microparticles. Values are expressed in units/mg weight of dried tissues as mean ± S.E.M. (n= 3-4). * $P < 0.05$ *vs*. NonSMPs.

Figure 6. Effects of nonseptic (NonSMPs) and septic (SMPs) microparticles on nitration of proteins. (A) Western blot showing tyrosine nitration in lungs. (B) Immunoblots from heart, lungs, liver, and kidneys were quantified by densitometric analysis. Data are representative of four separate blots, and the densitometry values are expressed in arbitrary units (A.U.) as mean ± SEM. * $P < 0.05$ vs. NonSMPs.