The Effects of Sepsis on Mitochondria

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Background. Sepsis is associated with mitochondrial dysfunction and impaired oxygen consumption, which may condition clinical outcome independent of tissue oxygenation. However, mitochondrial role in sepsis severity remains unknown. We aimed to characterize mitochondrial function in sepsis, establish its origin and cellular consequences, and determine its correlation with clinical symptoms and outcome.

Methods. Different markers of mitochondrial activity, nitrosative and oxidative stress, apoptosis, and inflammation were measured in peripheral blood mononuclear cells (PBMCs) and plasma of 19 septic patients and 20 controls. Plasma capacity to induce mitochondrial dysfunction was assessed in muscle mitochondria from 5 healthy individuals incubated with plasma of septic patients or controls.

Results. Despite unaltered mitochondrial mass and protein synthesis, enzymatic mitochondrial complexes I, III, and IV and oxygen consumption were significantly inhibited in sepsis. Septic plasma tended to reduce oxygen consumption of healthy mitochondria and showed significantly increased amounts of extracellular mitochondrial DNA and inflammatory cytokines, especially in patients presenting adverse outcome. Active nuclear factor kappa-light-chain enhancer of activated B cells (NFKB) was also significantly increased, together with nitric oxide, oxidative stress and apoptosis. Additionally, sepsis severity significantly correlated with complex I inhibition, NFKB activation and intercellular adhesion molecule expression.

Conclusions. A plasmatic factor such as nitric oxide, increased in inflammation and able to induce mitochondrial dysfunction, oxidative stress and apoptosis, may be responsible for cell damage in sepsis. Together with bacterial infection, leakage of mitochondrial DNA from damaged cells into circulation could contribute to systemic inflammatory response syndrome. Mitochondrial dysfunction and inflammation correlate with sepsis severity and outcome, becoming targets for supporting therapies.

Sepsis is the systemic inflammatory response syndrome (SIRS) secondary to bacterial infection [1]. It is a complex defense mechanism triggered by microbial antigens and mediated through cytokines and cells [2]. It is characterized by activation of the inflammation and coagulation systems, which can lead to generalized hypoperfusion, multiorgan failure, and death [3]. The risk factors currently associated with favorable or unfavorable outcome are poorly understood.

Cells of septic patients seem to be unable to maintain intermediate metabolism and, consequently, develop an energetic failure that may lead to cell death and threaten the patient's life [4]. Oxidative metabolism is highly energetic compared with anaerobic glucolysis, and thus the viability of high energy–dependent cells (especially neurons, myocytes, or hepatocytes) depends directly on continuous oxygen delivery. Many factors may limit the arrival of oxygen into the tissue of septic patients: pulmonary damage, reduction in preload and cardiac output due to increased permeability and vessel dilatation, decrease in left ventricular function due to impaired myocardic contractibility, and increased microthrombi formation due to increased erythrocyte deformation and local formation of platelets and leukocytes, among others.

Liquid expansion, transfusions, and inotrophic agents designed to improve oxygen arrival into tissues reportedly enhance the outcome of septic patients, albeit only in early stages [5]. Indeed, oxygen administration in advanced phases of the disease has been described as detrimental [6]. Direct measures of partial oxygen pressure in blood of subjects in septic shock have demonstrated that the oxygen amount is preserved, even increased, suggesting that oxygen consumption, but not oxygen availability, could be impaired [7, 8]. The hypothesis supporting the inability of septic cells to use the oxygen available has been called cytopathic hypoxia [9]. Different experimental models support an underlying cytopathic hypoxia by mitochondrial dysfunction. First, pyruvate dehydrogenase would be inhibited, leading to increased lactate production [10] and reduced mitochondrial energetic activity. A second mechanism would involve the activation of poly (adenosine diphosphate–ribose)polymerase-1 enzyme through DNA breakage (apoptosis), inflammation mediators [11], and reactive oxygen species (ROS), especially peroxinitrite (ONOO·) [12], which is considered a highly cytotoxic radical. The activation of this enzyme would reduce nicotinamide adenine dinucleotide, oxidized/nicotinamide adenine dinucleotide, reduced (NADH) content, leading to inhibition of mitochondrial respiratory chain (MRC) complex I (CI). Finally, endotoxins have been shown to promote inducible nitric oxide synthase activity and, consequently, increase the amount of nitric oxide (NO·) that directly binds to and limits MRC complex IV (CIV) [13]. Mitochondrial CIV is responsible for final oxygen reduction into water and, its inhibition could lead to mitochondrial dysfunction and oxidative energetic failure, even in an oxygen-rich atmosphere. Additionally, NO· can inhibit the mitochondrial adenosine triphosphate mitochondrial adenosine triphosphate/proton synthase (ATPase)/H^+ synthase responsible for energetic cell production [14]. Moreover, interaction of NO· with O_2^- generates $ONOO^-$ that is able to inhibit mitochondrial CI [15]. Adequate control of all these mechanisms could be more relevant for cell survival than correct tissular perfusion and oxygen delivery into tissues.

Most of these findings have been reported in experimental models. In humans there is little evidence of mitochondrial impairment in sepsis. One study performed in quadricep muscle tissue of severe septic patients established a relationship between NO· production, antioxidant depletion, adenosine triphosphate decrease, and MRC dysfunction, especially mediated by CI impairment [16]. Other studies have found differences on comparing respiratory and leg muscles [17, 18]. Nonetheless, perfusion is not guaranteed in muscle studies because of the septic process, and thus the results reported could be partially due to hypoxia, particularly in the development of shock. Additionally, muscle biopsy constitutes an invasive approach, making longitudinal follow-up in patients with unstable clinical conditions difficult.

Mitochondrial studies in peripheral blood mononuclear cells (PBMCs), particularly after setting the methodological conditions for mitochondrial CI activity measurement [19], are currently a good alternative to study mitochondrial dysfunction [20]. Circulating cells are not affected by an eventual hypoxemia and, because they participate in immune response, may exemplify the immune dysfunction underlying septic SIRS.

It has recently been shown that leakage of mitochondrial DNA (mtDNA) or mitochondrial peptides into the bloodstream in different diseases (trauma, cancer, or human immunodeficiency virus [HIV] chronic inflammation) [21–23] can activate the immune system and may contribute to SIRS and compromise organ function.

The aim of this study was to assess mitochondrial function in circulating PBMCs of patients in the initial stages of sepsis, in the absence of shock. As secondary objectives, we aimed to characterize the levels of plasmatic mtDNA, inflammatory cytokines, nitric oxide, oxidative stress, and apoptosis in these patients and determine their origin. The hypothesis is that disarrangement in these metabolic pathways may contribute to or be the result of a cell energetic failure that correlates with clinical manifestations and septic outcome.

MATERIAL AND METHODS

Patients

From March 2005 to November 2009, we included 19 patients consecutively admitted to the Hospital Clinic of Barcelona for infectious SIRS. Inclusion criteria for septic patients were the presence of an identifiable site of infection and evidence of SIRS without septic shock, as manifested by 2 of the following signs: temperature $>38°C$ or $<36°C$, heart rate >90 beats/min, respiratory rate >20 breaths/min or partial pressure of carbon dioxide in the blood ($PaCO_2$) <32 mmHg, and white blood cell count $>12 \times 10^9$ cells/L, $<4 \times 10^9$ cells/L, or $>10\%$ of immature forms.

Twenty healthy volunteers, matched by age and sex with septic patients, were included as controls for blood analysis. Additionally, for the isolation of mitochondria, 5 additional healthy volunteers undergoing hip replacement surgery were included for skeletal muscle collection.

Exclusion criteria for both patients and controls were the presence of a family history of primary mitochondrial disease, treatment with potential mitochondrial drugs [24], and septic shock. All individuals or their next of kin, in patients with altered consciousness, gave informed consent to be included in this protocol, which had been previously approved by the ethical committee of our hospital.

Samples
Plasma
Plasma was collected in ethylenediaminetetraacetic acid tubes from 20 mL of whole peripheral blood by centrifugation at 1500 g for 15 minutes and immediately stored at $-80°C$ until

analysis. Plasma was used for the quantification of NO·, mtDNA, and cytokine levels and the screening of potential inducers of mitochondrial dysfunction.

Peripheral Blood Mononuclear Cells

Once plasma was collected, PBMCs were isolated by Ficoll density gradient centrifugation [25]. PBMCs were used to measure mitochondrial, apoptotic, and oxidative stress markers; fresh cells were used to measure mitochondrial membrane potential and oxygen consumption, and frozen cells were used for the remaining measures.

Isolation of Mitochondria

Mitochondria were isolated from skeletal muscle of healthy controls according to the methodology of [26], and mitochondria were incubated with either septic or control plasma in order to search for potential plasmatic inducers of mitochondrial dysfunction able to impair oxygen consumption of healthy mitochondria.

Protein Content

All PBMCs or skeletal muscle parameters were normalized to protein content measured according to the Bradford protein-dye binding–based method [27].

Mitochondrial Markers

Mitochondrial Protein Synthesis

Mitochondrial protein synthesis was assessed by Western blot immunoquantification of the mitochondrial-encoded cytochrome c oxidase (COX) II subunit and the nuclear-encoded COX-IV subunit, both components of the mitochondrial CIV, normalized by the amount of β-actin protein to establish the relative COX-II or COX-IV abundance per overall cell protein [28].

Global and Specific MRC CI-Stimulated Oxygen Consumption

Global and specific MRC CI-stimulated oxygen consumption was measured by polarography using a thermostated Clark electrode at 37°C. Different oxidative activities were measured: the first was performed in intact cells as indicative of spontaneous and endogenous substrate consumption, and the second was undertaken in digitonin-permeabilized cells using specific electron donors and inhibitors of CI to specifically quantify CI-stimulated oxygen consumption. Oxidative activities were expressed as nanomoles of oxygen per minute and milligram of cellular protein [26, 29].

Enzymatic Activity of MRC Complexes I, III and IV

The enzymatic activity of MRC complexes I, III, and IV were measured by thermostatized spectrophotometry at 37°C according to the methodology of Rustin et al [26], slightly modified for CIV measurement in minute amounts of biological samples [30]. Measurement of CI activity required previous treatment of cells with triton and digitonin detergents to obtain specific mitochondrial NADH-dehydrogenase activity [19]. Enzymatic activities were expressed as nanomoles of consumed substrate or generated product per minute and milligram of protein [19, 26, 30].

Mitochondrial Content

Mitochondrial content was estimated by thermostatized spectrophotometry at 37°C with the measurement of citrate synthase (CS) activity [28] because CS is a Kreb cycle enzyme widely considered as a reliable marker of mitochondrial amount [26, 31–33].

Plasmatic Mitochondrial Dysfunction Inducer Analysis

Skeletal muscle mitochondria from healthy controls were incubated for 30 minutes at 37°C with plasma either from septic patients or healthy volunteers. After incubation we measured CI- or CIV-stimulated oxygen consumption by polarography to search for eventual plasmatic inducers of mitochondrial dysfunction.

Apoptotic Markers

Mitochondrial Membrane Potential

Flow cytometry was used to measure mitochondrial membrane potential by JC-1 staining [34]. The results were expressed as the percentage of PBMCs with depolarized mitochondria compared with the total amount of PBMCs analyzed and were interpreted as a marker of early apoptosis.

Caspase 3

The activation of this protein was determined by Western blot immunoquantification of cleaved caspase 3 proapoptotic protein normalized by the content of β-actin protein as a cell loading control. Chemoluminescence results were expressed as caspase 3/β-actin relative content and were interpreted as a marker of advanced apoptosis.

Inflammatory Markers

Plasmatic mtDNA Content

Extracellular mtDNA was isolated from 400 μL of filtered plasma (using a 0.22-μm filter pore to exclude circulating bacteria) through the QIAamp DNA Blood Mini Kit (Qiagen), as described elsewhere [21–23, 35]. A 235–base pair fragment of the mitochondrial-encoded *ND2* gene was quantified by SYBER-green and Roche rtPCR Technology [28] after testing specificity through melting temperature analysis and checking for no sequence homology with DNA found in any bacterial specie published on the basic local alignment search tool. Plasmatic mtDNA content was expressed as the absolute amount of *ND2* gene per milliliter of plasma [35].

Plasmatic Cytokines

Plasmatic cytokines were assessed with Luminex technology, allowing simultaneous measurement through internal curve quantification of either plasmatic interleukin (IL) 6, tumor necrosis factor (TNF) α and monocyte chemotactic protein 1(MCP-1) molecules or intercellular adhesion molecule (I-CAM) and vascular cell adhesion protein (V-CAM) cytokines.

Nuclear Factor Kappa-Light-Chain Enhancer of Activated B Cells

Nuclear factor kappa-light-chain enhancer of activated B cells (NF$_K$B) was assessed by Western blot immunoquantification of

phosphorylated and active NF$_K$B protein expression normalized by the content of total NF$_K$B protein as a cell loading control. Chemoluminescence results were expressed as phosphorylated/total NF$_K$B relative content and were interpreted as a marker of inflammation, cellular stress, and survival.

Nitrosative and Oxidative Stress Markers

Plasmatic NO·

Plasmatic NO· was indirectly measured by the spectrophotometric measurement at 540 nm of plasmatic nitrate and nitrite amount (both products of NO· metabolism) using the Griess assay adapted to plasma samples [36] in the Lipid-LPL Unit of the University of Barcelona (Titertek Multiskan PLUS MKII) [37].

Lipid Peroxidation

Lipid peroxidation was measured as an indicator of oxidative damage of ROS in cellular lipid compounds using the Oxys Research kit of Deltaclon by spectrophotometric measurement of malondialdehyde (MDA) and 4-hydroxyalkenal (HAE), both products derived from fatty acid peroxide decomposition, normalized by protein content and expressed as the concentration of MDA and HAE (in micromoles per liter) per milligram of cell protein [38].

Statistical Analysis

Mitochondrial, inflammatory, apoptotic, nitrosative, and oxidative stress markers were compared between septic and healthy subjects. The results were expressed as means ± standard errors of the mean or as percentages of decrease or increase with respect to healthy controls. Differences between groups were determined with the nonparametric Mann–Whitney test for independent measures and correlation between quantitative parameters with the nonparametric Spearman analysis. The cutoff for significance (P value) was set at .05.

RESULTS

The main characteristics of the septic patients at inclusion are shown in Table 1. The patients included were predominantly female (15/19) with a mean age of 64 years. The primary sites of infection were thoracic (6/19), urinary (5/19), abdominal (3/19), and skin (3/19). At inclusion, the patients were in the initial stages of SIRS and presented a mean simplified acute physiology score (SAPS II) of 45.5 and a sequential organ failure assessment (SOFA) value of 7.6, corresponding to moderate to severe sepsis.

Mitochondrial PBMC markers showed unaltered protein expression for MRC subunits compared with controls, considering either mitochondrial-encoded (COX-II) or nuclear-encoded (COX-IV) proteins. The number of mitochondria in PBMCs of septic patients was also not modified (data not shown). On the contrary, PBMCs of septic patients compared with healthy subjects showed a reduction of 32% in CI function ($P < .05$), a decrease of 42% in CIII activity ($P < .05$), and an inhibition of

Table 1. Clinical and Epidemiological Characteristics of the Patients Included in the Study

| Characteristic	Value
Age, mean years ± SD	64 ± 18
Sex, No. female/No. male	15/4
Site of infection, No.	
Thoracic	6
Urinary	5
Abdominal	3
Skin	3
Other	2
SAPS II in first 24 h[a]	45.5 ± 13.3
SOFA[a]	7.6 ± 3.2
Sepsis outcome, No. (%)	
Death[b]	6 (32)
Survival	13 (68)

Abbreviations: SAPS II, simplified acute physiology score; SD, standard deviation; SOFA, sequential organ failure assessment.

[a] Both the SAPS II and SOFA scales classify sepsis severity from null (0 score) to severe forms of the disease (maximum score is 60 for the SAPS II scale and 10 for the SOFA). Individual values in the SAPS II and SOFA scales were calculated for each patient, at study inclusion, specifically punctuating all physiological measures indicative of sepsis development included in both measures.

[b] Patients who died had comorbidities with either lymphoma, myeloblastic syndrome, metastatic lung cancer, Chronic obstructive lung disease stage IV, or Alzheimer dementia.
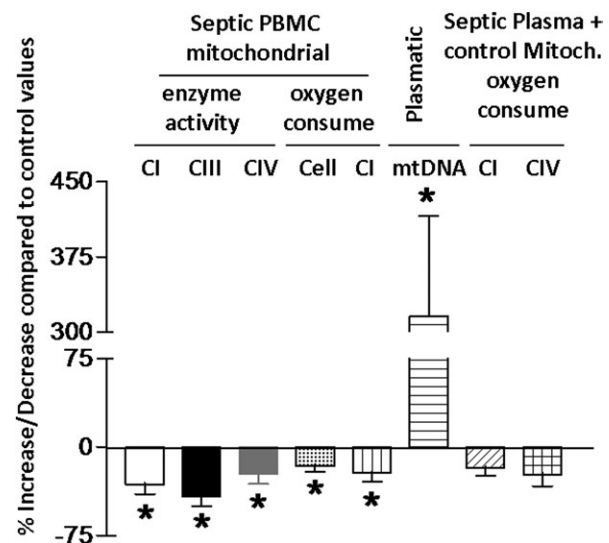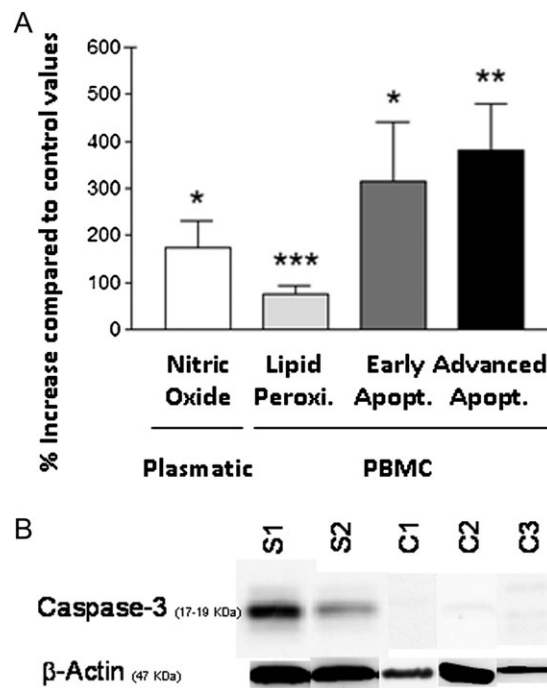

Figure 1. Mitochondrial markers in peripheral blood mononuclear cells (PBMCs) of septic patients and in mitochondria of healthy individuals incubated with plasma of septic patients, with respect to controls. Bars represent the percentage of increase/decrease in septic patients with respect to controls. Abbreviations: Cell, spontaneous cell (oxygen consumption); CI, mitochondrial respiratory chain complex I; CIII, mitochondrial respiratory chain complex III; CIV, mitochondrial respiratory chain complex IV; mitochon, mitochondria; mtDNA: mitochondrial DNA. *$P < .05$ between cases and controls.
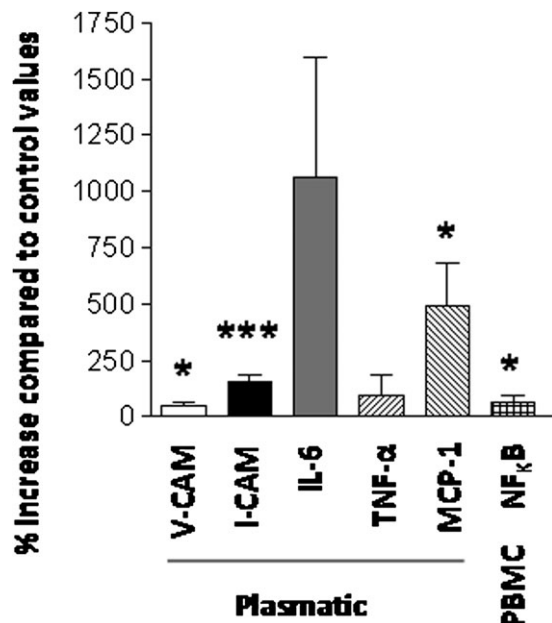
Figure 2. Inflammatory markers of plasmatic cytokine expression and peripheral blood mononuclear cells (PBMCs) NF$_K$B activation in septic patients with respect to controls. Bars represent the percentage of increase in septic patients with respect to controls. Abbreviations: I-CAM, intercellular adhesion molecule; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; NF$_K$B, nuclear factor kappa-light-chain enhancer of activated B cells; TNFα, tumor necrosis factorα; V-CAM, vascular cell adhesion protein. * $P < .05$; ***$P < .001$ between cases and controls.


Figure 3. Nitrosative, oxidative, and apoptotic markers in plasma and peripheral blood mononuclear cells (PBMCs) of septic patients with respect to controls. *A*, Plasmatic nitric oxide levels and PBMC oxidative stress and early (mitochondria membrane potential) or advanced (active caspase 3/β-actin) apoptosis development. Bars represent the percentage of increase in septic patients with respect to controls. Apopt., apoptosis; Lipid Peroxi., lipid peroxidation. *$P < .05$; **$P < .005$; ***$P < .001$ between cases and controls. *B*, Western blot analysis of advanced apoptosis (active caspase 3 amount/β-actin) in PBMCs of septic patients. Note increased active caspase 3 in PBMCs of septic patients with respect to controls for similar amounts of β-actin signal.

mitochondrial CIV of 23% ($P < .05$) (Figure 1). Consequently, the capacity of PBMCs of septic patients to consume oxygen was also significantly impaired for either endogenous substrates (spontaneous cell oxygen consumption, 16% decrease; $P < .05$) or CI-stimulated oxygen consumption (22% decrease; $P < .05$) (Figure 1). Additionally, plasma of septic patients incubated with mitochondria from healthy individuals tended to decrease the oxidative capacity of control mitochondria to consume oxygen, although not significantly (18% decrease in CI-stimulated oxygen consumption and 23% decrease in CIV-stimulated respiration, not significant [NS]) (Figure 1).

Inflammatory markers increased in plasma of septic patients compared with controls. The specific increase in proinflammatory cytokines was 46% for V-CAM ($P < .05$), 155% for I-CAM ($P < .001$), 1062% for IL-6 (NS), 98% for TNF-α (NS) and 494% for MCP-1 ($P < .05$) (Figure 2). Moreover, the amount of circulating free mtDNA was significantly increased in plasma of septic patients with respect to controls (316% increase; $P < .05$). Plasmatic mtDNA content significantly and positively correlated with V-CAM and TNF-α expression ($P < .05$; data not shown). Additionally, phosphorylated and transcriptional active NF$_K$B increased 68% in PBMCs of septic patients compared with healthy volunteers ($P < .05$) (Figure 2). Nitrosative and oxidative stress markers (nitric oxide amount and lipid peroxidation levels) were

also increased 174% and 76%, respectively, in PBMCs of septic subjects compared with controls ($P < .05$ and $P < .001$) (Figure 3A).

All apoptotic markers were also significantly increased. The percentage of cells with depolarized mitochondria, indicative of early apoptosis, increased 315% in septic PBMCs compared with controls ($P < .05$) and the expression of active (cleaved) caspase 3 protein with respect to β-actin amount, indicative of advanced apoptosis, increased 380% ($P < .005$) (Figure 3A and 3B).

Sepsis severity measured using the SAPS scale negatively correlated with PBMC CI enzymatic function ($R^2 = 0.715$; $P < .001$) (Figure 4). Additionally, CI-stimulated oxygen consumption in PBMCs of septic patients and mitochondria from control individuals incubated with septic plasma were positively correlated ($R^2 = 0.300$; $P < .05$) (Figure 4). Furthermore, sepsis severity was positively correlated with NF$_K$B activation and I-CAM expression (both $P < .05$) (Figure 4). Finally, V-CAM, MCP-1, and IL-6 cytokine expression and lipid peroxidation were abnormally increased in septic patients presenting a fatal
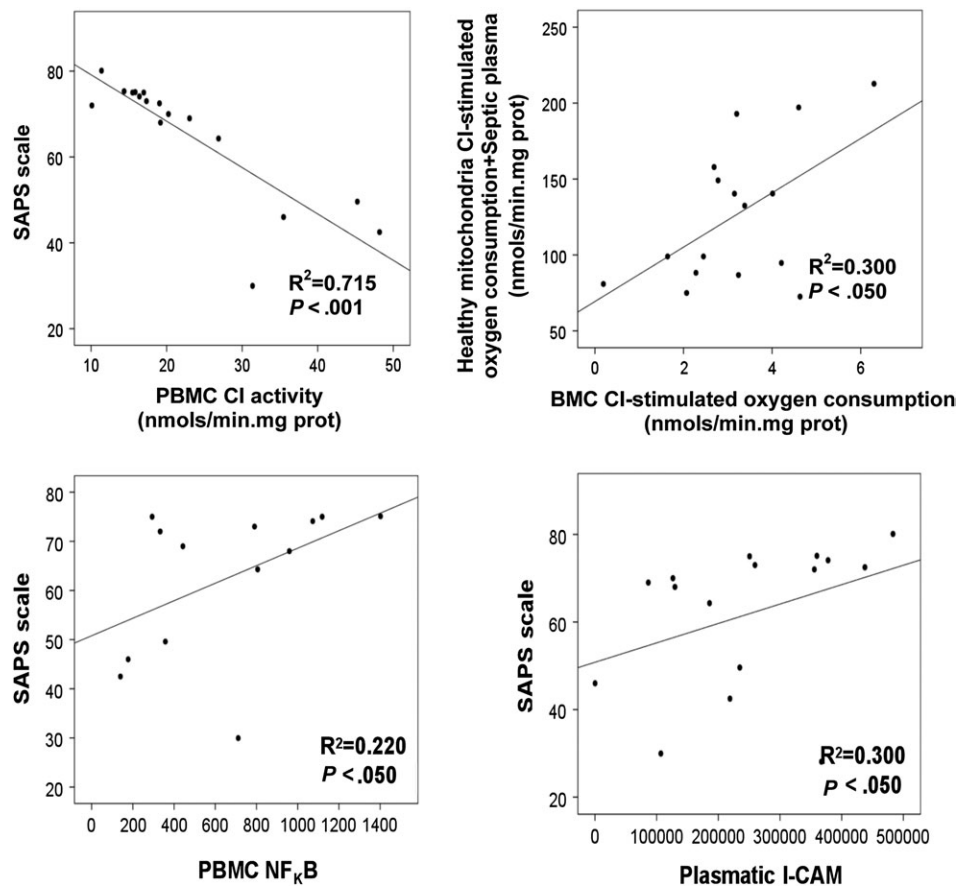
Figure 4. Correlations among mitochondrial or inflammatory markers and clinical parameters.

outcome, compared with patients with a favorable disease evolution ($P < .05$; all cases) (Figure 5).

DISCUSSION

Our findings support the presence of general mitochondrial dysfunction in patients in early stages of sepsis, in the absence of shock or multiple organ failure. Such mitochondrial impairment was first theoretically suggested because of clinical evidence of impaired oxygen extraction in sepsis, despite apparent correct tissular perfusion. Mitochondrial dysfunction could be interpreted as a normal physiological response to metabolic changes occurring in sepsis because metabolic slow-down has been suggested to play a protective role [39]. However, here we describe a multitude of simultaneous disarrangements that suggest a primary and pathological role for mitochondrial dysfunction.

In PBMCs of septic patients we found that mitochondrial protein synthesis was unaltered, despite a previous report showing altered protein expression in patients with sepsis-induced multiple organ failure [40]. The mitochondrial number was also not altered in our series of septic patients. However, we found a global mitochondrial dysfunction affecting different MRC enzymes (CI, CIII, and CIV) and oxygen consumption (spontaneous or CI stimulated) responsible for energy supply. Mitochondrial dysfunction of CI and CIV has been reported elsewhere in muscle tissue of patients with septic shock and multiorgan failure [16, 40]. Our results confirm that mitochondrial dysfunction is present in the PBMCs of septic patients regardless of the absence of these clinical manifestations. Consequently, mitochondrial dysfunction seems to be a primary event in sepsis, whereas mitochondrial protein synthesis deregulation may be exclusively attributed to advanced stages of sepsis in which multiorgan failure is frequent.

Mitochondria are the respiratory and energetic centers of cells. However, mitochondrial dysfunction enhances ROS production, especially when CI and CIII are impaired. ROS are highly unstable molecules that attack cellular structures, including nucleic acids, lipids, carbohydrates, and proteins, causing oxidative stress damage that can lead cells to apoptosis. Although impaired oxidative metabolism has been suggested elsewhere to play a role in sepsis through increased superoxide dismutase activity [40], our study is the first to correlate increased oxidative stress with an unfavorable outcome. Additionally, apoptotic and NF_KB levels were also increased in cells of septic patients.
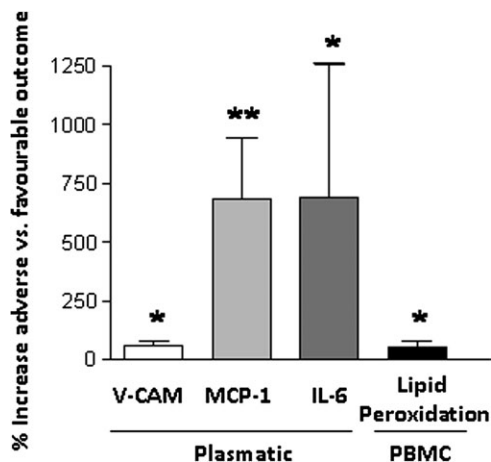
Figure 5. Markers of plasmatic inflammation and peripheral blood mononuclear cells (PBMCs) oxidative stress depending on sepsis outcome. Bars represent the percentage of increase in septic patients with fatal outcome with respect to septic patients presenting a favorable evolution. Abbreviations: I-CAM, intercellular adhesion molecule; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; V-CAM: vascular cell adhesion protein. *$P < .05$ between septic patients with a favorable or fatal septic outcome; ** $P < .005$.

Apoptosis, especially when cell death involves immune system effectors, may be particularly relevant in septic patients that require effective function of their defense cells.

Our results suggest that, in the context of sepsis, mitochondrial dysfunction may increase oxidative stress, which could, in turn, enhance apoptosis. All of these physiopathological processes may be linked and may be primarily caused by mitochondrial dysfunction, as suggested elsewhere [41]. However, the specific mechanisms that underlie mitochondrial dysfunction and derived oxidative and cell damage have only been theoretically suggested.

Other studies have demonstrated that mitochondrial dysfunction is inducible in healthy cultured cells with plasma of septic patients [42]. We have confirmed this finding in isolated mitochondria with CI- and CIV-stimulated oxygen consumption deficiency. Nonetheless, our results were not statistically significant, perhaps owing to the small sample size or to inappropriate incubation conditions. However, we show, for the first time, a positive correlation between the mitochondrial lesion induced by septic plasma in mitochondria from healthy controls and the mitochondrial damage present in PBMCs from the same septic patients. This finding confirms the existence of a plasmatic agent able to induce the systemic mitochondrial lesion present in cells of septic patients and potentially responsible for interference in ROS metabolism and, consequently, for triggering apoptosis.

We found abnormally increased amounts of NO· and cytokines in the plasma of septic patients. These cell messengers are produced as a consequence of bacterial infection; additionally, NO· is a mediator of inflammatory response secondary to infection. NO· is formed by constitutive and inducible NO synthases in response to inflammation, and although vital for cell life in standard circumstances, an increase in NO· concentrations over certain physiological ranges may induce oxidative stress and mitochondrial dysfunction. Inhibition of CIV has been suggested to be caused by direct NO· binding to the CIV core. Moreover, NO· interaction with O_2^- leads to the formation of ONOO·, which has also been suggested to bind and inhibit mitochondrial CI. We observed inhibited CIV function in PBMCs of septic patients and, additionally, for the first time, we observed that the inhibition of CI was directly related to sepsis severity determined with the SAPS scale. Consequently, mitochondrial dysfunction may be responsible, at least in part, for the clinical symptoms and evolution of sepsis, which seem to be caused by some plasmatic factor similar to NO· presenting dual mitotoxic and cytotoxic effects. Our study corroborates in blood cells (not susceptible to hypoperfusion) the results of Breadley and Singer performed in muscle tissue [39], suggesting that exacerbated immunological response in sepsis may enhance NO· above physiological ranges [43], causing the derived mitochondrial, oxidative, and cell lesions. Thus, the study and potential treatment of mitochondrial dysfunction and NO· metabolism could be of interest in the clinical management of sepsis.

Furthermore, extracellular free mtDNA levels were increased in septic patients, suggesting that immune activation and SIRS may not be exclusively restricted to infectious pathogens but also secondarily caused by circulating mitochondrial antigens. Such mitochondrial compounds (especially mtDNA or formyl peptides), also called damage-associated molecular patterns (DAMPs), were first described to cause inflammatory responses to injury in cases of trauma, some cancers, or chronic HIV inflammation [21–23] due to analog immune activation to that caused by microbial pathogen-associated molecular patterns (PAMPs). In sepsis, mitochondrial DAMPs could probably be released into the bloodstream by leakage from cells damaged through mitochondrial, apoptotic, or inflammatory means.

Patients presenting severe acute symptoms and adverse clinical outcome showed increased levels of mitochondrial dysfunction (CI activity), oxidative stress, and inflammatory response (I-CAM, V-CAM, MCP-1, IL-6, and NF$_K$B levels). This observation was reported elsewhere for an increase in IL-6 [44] and other proinflammatory cytokines [45], suggesting that all these mechanisms of physiological response to bacterial infection could influence the development of sepsis and patient survival and are, together with NO·, prognostic factors of disease progression. Further studies interfering in the NF$_K$B transduction pathway or ROS metabolism could help discard alternative mechanistic explanations.

A limitation of the present study is that PBMCs may be poorly representative of postmitotic tissues more directly related to mortality and multiorgan failure in sepsis, such as heart, liver, brain, or muscle. PBMCs are immune system effectors that

become activated during infection, and their eventual adaptation to energy supply may differ from cells that exclusively rely on mitochondria to meet their energetic demands. On the other hand, PBMCs are easy to obtain, do not require invasive approaches, are not susceptible to the hypoperfusion found in muscle biopsies of septic patients, and have been validated elsewhere in the study of many other mitochondrial disorders.

If the inability of septic patients to consume oxygen is due not to hypoxia (lack of oxygen transport into tissues) but rather to energetic and metabolic failure of mitochondria (cytopathic hypoxia and consequent oxidative damage that could lead cell to apoptosis), clinical measures aimed at preserving organ and tissue function should be modified to potentiate mitochondrial function, in addition to providing adequate perfusion and oxygenation.

Notes

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