

Preclinical pulmonary capillary endothelial dysfunction is present in brain dead subjects

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ABSTRACT

Pulmonary endothelium is a major metabolic organ affecting pulmonary and systemic vascular homeostasis. Brain death (BD)-induced physiologic and metabolic derangements in donors' lungs, in the absence of overt lung pathology, may cause pulmonary dysfunction and compromise post-transplant graft function. To explore the impact of BD on pulmonary endothelium, we estimated pulmonary capillary endothelium-bound (PCEB)-angiotensin converting enzyme (ACE) activity, a direct and quantifiable index of pulmonary endothelial function, in eight brain-dead patients and ten brain-injured mechanically ventilated controls. No subject suffered from acute lung injury or any other overt lung pathology. Applying indicator-dilution type techniques, we measured single-pass transpulmonary percent metabolism (%M) and hydrolysis (v) of the synthetic, biologically inactive, and highly specific for ACE substrate ³H-benzoyl-Phe-Ala-Pro, under first order reaction conditions, and calculated lung functional capillary surface area (FCSA). Substrate %M (35 ± 6.8%) and v (0.49 ± 0.13) in BD patients were decreased as compared to controls (55.9 ± 4.9, *P* = 0.033 and 0.9 ± 0.15, *P* = 0.033, respectively), denoting decreased pulmonary endothelial enzyme activity at the capillary level; FCSA, a reflection of endothelial enzyme activity per vascular bed, was also decreased (BD patients: 1,563 ± 562 mL/min vs 4,235 ± 559 in controls; *P* = 0.003). We conclude that BD is associated with subtle pulmonary endothelial injury, expressed by decreased PCEB-ACE activity. The applied indicator-dilution type technique provides direct and quantifiable indices of pulmonary endothelial function at the bedside that may reveal the existence of preclinical lung pathology in potential lung donors.

Key Words: angiotensin converting enzyme, brain death, pulmonary endothelium

Lung transplantation is often the only available treatment option for patients with end-stage vascular and other lung disease. However, despite the advances in surgical techniques and pharmacologic management, a significant proportion of patients do not benefit from transplantation, due to severe early allograft dysfunction; this may account for the death of 20% of recipients in the first few weeks after transplantation.^[1] Although several factors may contribute to the adverse prognosis of lung transplant recipients, there is strong evidence that preclinical lung injury is already present in donor lungs before their retrieval.^[2] Potential lung donors are generally patients admitted in the Intensive Care Unit (ICU), who progress to brain death (BD) following irreversible cessation of brainstem function;^[3,4]

such patients are considered at high risk for development of lung injury due to trauma, mechanical ventilation, aspiration, or infection. Additionally, the process of BD itself can damage the lung directly and jeopardize its function post-transplantation.^[5]

BD may cause pulmonary dysfunction secondary to α -adrenergic stimulation and hemodynamic derangements of the pulmonary capillaries.^[4,6] Evidence also suggests that BD results in a systemic inflammatory response by the release of potent proinflammatory mediators into the systemic circulation^[7] that could induce preclinical lung

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injury and undermine graft survival.^[2] However, human studies have thus far focused on the alveolar epithelium and capillary barrier function; direct *in vivo* evidence on the contribution of pulmonary endothelium in such a BD-induced subtle lung injury is still missing.^[3,6]

Pulmonary endothelium (PE) is a major metabolic organ that warrants the maintenance of systemic and pulmonary circulation homeostasis.^[8,9] PE may be affected by either the BD-induced inflammatory response and/or the above mentioned hemodynamic perturbations and shear stress; the latter have been shown to upregulate various endothelial inflammatory pathways,^[6] including reactive oxygen species generation, nuclear factor- κ B (NF- κ B) activation, and upregulation of adhesion molecules and pro- or anti-inflammatory cytokines.^[10-13]

To investigate the role of BD as a factor causing preclinical lung injury, we estimated pulmonary endothelial function in BD subjects. We hypothesized that BD may induce pulmonary endothelial dysfunction, denoted by pulmonary endothelial angiotensin converting enzyme (ACE) activity reduction, as a result of the BD-triggered inflammatory response. To this end, we compared pulmonary capillary endothelium-bound-ACE (PCEB-ACE) activity and plasma inflammatory mediator levels in BD patients and brain-injured mechanically ventilated controls. ACE is expressed as an ectoenzyme on the PE surface, and PCEB-ACE activity may be measured by means of indicator dilution techniques that allow quantifiable assessments of (1) the enzyme activity at the capillary endothelial level and (2) the functional capillary surface area (FCSA) which is available for reaction.^[14-17] Early PCEB-ACE activity reduction has been documented in various animal models of acute lung injury (ALI) as well as in patients with ALI and acute respiratory distress syndrome (ARDS).^[9,16,18,19] In this study, we found that PCEB-ACE activity in BD patients with no evidence of ALI or other overt lung pathology was reduced compared to mechanically ventilated brain-injured patients with functioning brainstem.

MATERIALS AND METHODS

Study population

The study was conducted in compliance with the Declaration of Helsinki and its protocol was reviewed and approved by our Institutional Ethics Committee. Informed written consent was obtained from subjects' next of kin. Eighteen patients were enrolled in the study; they were all hospitalized in a mixed (i.e., medical and surgical) ICU of a general hospital. All patients had catheters placed in either the subclavian or the internal jugular vein and in the radial artery, as part of their routine treatment. Eight patients had developed BD (BD group), and ten patients who suffered from brain trauma or injury but never

developed BD served as controls. Patients' traumatic or medical injuries were diagnosed by neurologists and/or neurosurgeons based on computerized tomographies of the brain. No subject had thoracic or lung trauma, ALI, or any other overt lung pathology. Descriptive data consisting of demographics, diagnosis, clinical and laboratory data, and lung injury score (LIS)^[20] were recorded. Chest X-ray (CXR) score, a LIS component, was independently measured. CXR score ranges from 0 to 4, depending on the absence (0) or presence of alveolar consolidations confined to one (1) up to all four lung quadrants (4).^[20] CXR scoring was performed by two "blind" nonstudy-related intensivists. Most BD subjects exhibited mild elevations of aspartate aminotransferase (AST), and two exhibited mild elevations of alanine aminotransferase (ALT) in serum; no BD patient exhibited elevated circulating bilirubin or creatine levels. Thus no BD subject suffered from overt liver or renal failure.

BD diagnosis had been confirmed when an irreversible catastrophic structural brain lesion resulted in unresponsiveness to noxious pain stimuli and to abolition of brainstem reflexes (papillary light responses, corneal reflexes, vestibulo-ocular tests, tracheobronchial stimulation) in the absence of hypothermia, metabolic or electrolyte disturbances, and depressant drugs. Testing for apnea was performed twice, with 24 hours in between, using previously described guidelines after all other prespecified brain-death criteria had been fulfilled.^[21] Patients were announced brain dead by a medical team that included a neurologist or a neurosurgeon, an anesthetist, and the treating attending intensivist, in compliance with Greek regulations.

Laboratory measurements

Immediately prior to PCEB-ACE activity measurements, venous blood was obtained by venous puncture into EDTA-containing tubes and was immediately placed on ice before centrifugation at $1000 \times g$ at 4°C for 20 minutes. Plasma samples were then aliquoted and stored at -80°C until processed. Tumor necrosis factor- α (TNF- α), interleukin (IL)-6, and -8 concentrations were measured in duplicate by flow cytometry using cytometric bead array (CBA) technology after staining with monoclonal antibodies and passage through a FACSCalibur flow cytometric device (Becton Dickinson, Cockeysville, Md., USA). The lowest limits of detection were as follows: 1.1 pg/mL for TNF- α ; 1.7 pg/mL for IL-6; and 2.5 pg/mL for IL-8.^[22] C-reactive protein (CRP) was measured using an immunoturbometric assay (Tina-quart C-reactive protein, Roche Diagnostics GmbH, Mannheim, Germany). S-100b protein was measured by an immunoluminometric technique with a commercially available kit (LIA-mat Sangtec 100, AB Sangtec Medical, Bromma, Sweden).^[23] Procalcitonin (PCT) levels were determined by means of a specific and ultrasensitive immunoluminometric assay (Liaison Brahms procalcitonin, Diagnostica, Berlin, Germany).

Determination of PCEB-ACE activity

Determination of PCEB-ACE activity in BD subjects and controls was performed by means of indicator-dilution type techniques that have been described in detail elsewhere.^[15] We estimated the single pass transpulmonary utilization of the radiolabelled synthetic, hemodynamically inactive, and specific for ACE substrate ³H-benzoyl-Phe-Ala-Pro (³H-BPAP) by PCEB-ACE under first-order reaction conditions and calculated related kinetic parameters. Briefly, a 1.3 mL bolus of normal saline solution containing 17 μ Ci of ³H-BPAP; (22.2 Ci/mmole) was injected through the distal port of a central vein catheter and was distributed through the pulmonary vascular bed. Simultaneously, effluent arterial blood was withdrawn via a radial artery catheter by means of a peristaltic pump into a fraction-collector (1.2 mL blood/tube, 16 tubes). Blood was collected into 1.75 mL of normal saline containing 5 mM EDTA and 6.8 mM 8-hydroxyquinoline 5-sulfonic acid to prevent further activity of ACE in blood, and heparin 1,000 IU/L ("stop" solution). Four additional tubes containing 1.75 mL of "stop" solution, 1.2 mL of blood withdrawn before isotope injection, and 0.02 mL of the isotope mixture were used to calculate the amount of administered radioactivity.

After centrifugation of the blood samples (3,000 revolutions/minute for 10 minutes), 0.5 mL of the supernatant was transferred into a scintillation vial, and total ³H radioactivity was measured in 5 mL Ecoscint (National Diagnostics, Atlanta, Ga., USA). For determination of the radioactivity associated with metabolites, another 0.5 mL of the supernatant was transferred into a separate vial containing 2.5 mL HCL (0.12 N). Three mL of Toluene Scintillator (Packard, Meriden, Ct., USA) was added, samples were mixed, and radioactivity was measured 48 hours later. In this way, ~70% of the ³H BPAP metabolite ³H-benzoyl-Phe and < 10% of the parent ³H-BPAP were extracted in the organic phase of the mixture (toluene). The precise values were calculated by identically processing separate tubes containing substrate or previously synthesized product.^[15,16]

Estimates of PCEB-ACE activity included single pass ³H-BPAP percent metabolism (%M), transpulmonary hydrolysis (v), and functional capillary surface area (FCSA), originally termed A_{\max}/K_m .^[15]

$$\% M = 100 \times \{([S_0] - [S])/([S_0] \times (1 - nrf))\} \quad (1)$$

$$v = \ln \{(1 - nrf)/(([S]/[S_0]) - nrf)\} = [E] \times t_c \times k_{cat}/K_m \quad (2)$$

$$FCSA = E \times k_{cat}/K_m = PPF \times v \quad (3)$$

with $[S_0]$ and $[S]$ being the initial and final substrate concentrations, respectively, in the effluent arterial plasma

in dpm/mL, nrf being the nonreactive fraction of cis-BPAP (7%), and $[E]$, t_c , k_{cat} , K_m , E and PPF being the enzyme concentration available for reaction, capillary transit time (i.e., enzyme-substrate reaction time), catalytic rate constant, Henri-Michaelis-Menten constant, total enzyme mass available for reaction, and pulmonary plasma flow, respectively.^[15,17,18] Cardiac output (CO) was calculated as previously described, and PPF was calculated as $= CO \times (1 - \text{Hematocrit})$.^[18] Substrate hydrolysis (v) and %M reflect ACE activity per capillary, whereas FCSA (A_{\max}/K_m) reflects ACE activity per vascular bed.^[17-19]

Statistical analysis

Group data are presented as mean \pm SEM. For normally distributed data the Student's *t*-test was used. For non-normally distributed data, comparisons were performed using the Mann-Whitney U-test. Fisher's exact test was used for gender comparison. Spearman's rank correlation coefficients (r_s) were calculated to describe the relationships between the quantitative variables. In all analyses, two-tailed *P*-values < 0.05 were considered significant.

RESULTS

Patients' characteristics

Patient demographics, as well as clinical and laboratory data obtained on the day of PCEB-ACE activity determination, are shown in Table 1. Eight patients (six men) had developed BD and 10 patients (nine men) served as controls. All 18 patients had been mechanically ventilated for similar time periods, and with the same mode (assist control ventilation) and tidal volumes (8 mL/Kg). The diagnoses of BD were: Spontaneous ($n = 2$) or traumatic ($n = 3$) subarachnoid hemorrhage and spontaneous ($n = 2$) or traumatic ($n = 1$) intracerebral hemorrhage. Controls suffered brain trauma without deterioration to BD. There were no statistical differences between the groups in sex, age, or time on mechanical ventilation. No patient suffered from ALI or other overt lung pathology as denoted by PO_2/FIO_2 values and their chest X-rays (Table 1).

Table 1: Demographic and clinical data of the study patients

	Controls (n=10)	BD (n=8)
Age (years)	33.3 \pm 3.6 (20-52)	34.6 \pm 3.5 (19-54)
PO_2/FIO_2 (mmHg)	390 \pm 22	420 \pm 44
PEEP (cmH ₂ O)	0	0.13 \pm 0.1 (0-1)
CXR score	0	0
Days on MV	3.3 \pm 0.6 (1-5)	3.5 \pm 0.7 (1-7)
CO (L/min)	7.1 \pm 0.47	4.21 \pm 0.6*
PPF (L/min)	4.99 \pm 0.4	2.93 \pm 0.48*

Data are presented as means \pm SEM; **P*<0.05 from control group; **BD**: brain death; **PEEP**: positive end expiratory pressure; **CXR score**: chest X-ray score (calculated as in lung injury score-LIS,^[20] i.e., number of quadrants showing alveolar consolidation on chest roentgenogram); **Days on MV**: days on mechanical ventilation before measurement; **CO**: cardiac output; **PPF**: pulmonary plasma flow

BD induces pulmonary endothelial ACE activity reduction

ACE substrate utilization was reduced in BD patients: BPAP %M ($35 \pm 6.8\%$) and v (0.49 ± 0.13) were decreased in BD subjects, as compared to controls (55.9 ± 4.9 , $P = 0.033$ and 0.9 ± 0.15 , $P = 0.033$, respectively; Figures 1A and B).

Functional capillary surface area is decreased in BD patients

FCSA available for BPAP hydrolysis was decreased in BD patients ($1,563 \pm 562$ mL/min) as compared to controls ($4,235 \pm 559$ mL/min, $P = 0.003$; Fig. 2).

BD is associated with increased circulating inflammatory parameters

Circulating CRP, S-100b, and PCT were higher in plasma of BD subjects as compared to controls (Table 2). In addition, plasma levels of cytokines TNF- α and IL-6 were increased in BD as compared to controls, while no difference was noted in plasma IL-8 levels between the two groups (Table 2). In our population, neither substrate %M or v , nor FCSA were correlated with any of the aforementioned inflammatory indices.

DISCUSSION

Lung or lung-heart transplantation may be the life-saving intervention for patients with end-stage lung disease,

including subjects with pulmonary arterial hypertension that deteriorate despite adequate specific treatment. Severe early allograft dysfunction is a major complication that may be related, among others, to existing preclinical injury in the donor lung. In that respect, there is evidence that lung injury sustained very early following BD may compromise post-transplant graft function.^[2] It is thus necessary to further improve our means of detecting the presence of such pathology. To the best of our knowledge, this study provides the first direct in vivo evidence, obtained at patients' bedsides, that pulmonary endothelial dysfunction is present in BD subjects with no overt lung pathology.

Endothelial integrity in peripheral organs of brain-dead donors has more recently gained considerable attention in relation to potential harmful effects of BD on donor organ quality. Animal and human studies have shown that under conditions of hemodynamic instability, such as the ones that follow BD, vascular endothelial cells are susceptible to shifts in mechanical forces and shear stress,^[24] or systemic inflammatory insults.^[25] Endothelial dysfunction is among the earliest features occurring under and contributing to lung injury pathogenesis, as evidenced among others by the altered expression of circulating endothelial-specific proteins, soluble adhesion molecules, and endothelial barrier alterations.^[8,9] It should be noted, however, that the aforementioned indices of pulmonary endothelial injury are either surrogate or have been estimated in ex vivo systems. In contrast, estimating PCEB-ACE activity by means of indicator-dilution type technique provides a direct, sensitive, and quantifiable means of assessing pulmonary endothelial function at the bedside in humans.^[8,9,14-16]

PCEB-ACE is an ectoenzyme uniformly distributed along the luminal pulmonary endothelial surface with its catalytic site exposed to the blood stream, thus allowing interactions with bloodborne substrates and inhibitors without requiring the time and energy expense that would be needed by interactions with a cytosolic enzyme. Due to the very high

Table 2: Laboratory data of the study patients

	Controls (n=10)	BD (n=8)
CRP (mg/dL)	10.4 \pm 1.2	20.1 \pm 4.4*
s100b (μ g/L)	0.38 \pm 0.1	1.65 \pm 0.6*
PCT (ng/mL)	0.38 \pm 0.16	6.87 \pm 2.5*
TNF- α (pg/mL)	2.21 \pm 0.777	10.06 \pm 3.1*
IL-8 (pg/mL)	90.24 \pm 17.05	56.35 \pm 14.66
IL-6 (pg/mL)	98.44 \pm 9.3	345.84 \pm 114.7*

Data are presented as means \pm SEM; * $P < 0.05$ from control group; **BD**: brain death; **CRP**: C-reactive protein; **PCT**: procalcitonin; **TNF**: tumour necrosis factor; **IL**: interleukin

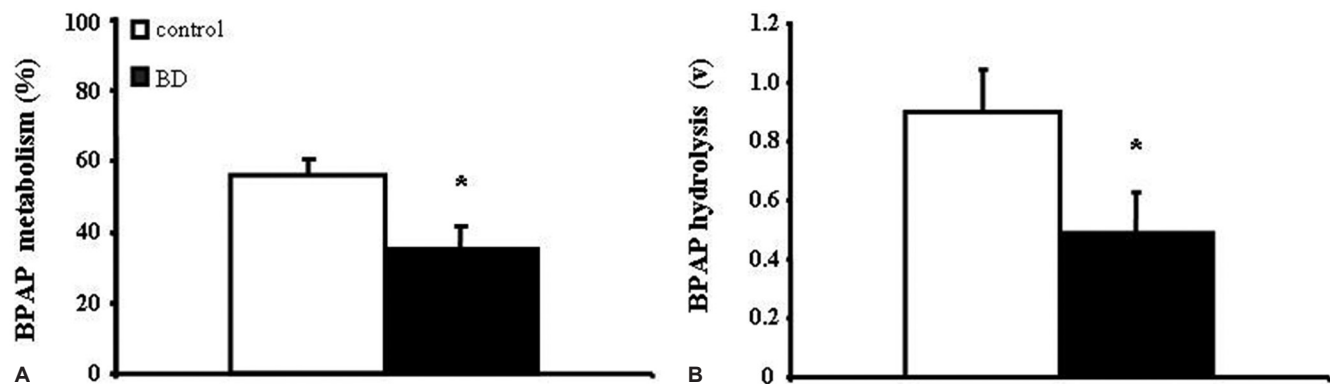


Figure 1: Brain death is associated with pulmonary endothelial ACE activity reduction, as compared to brain-injured controls. Substrate (BPAP) percent metabolism (%M; Panel A) and transpulmonary hydrolysis (v ; Panel B), both reflecting enzyme activity per capillary, were decreased in BD subjects ($n = 8$), as compared to controls ($n = 10$). Data are presented as means \pm SEM, * $P < 0.05$ from controls

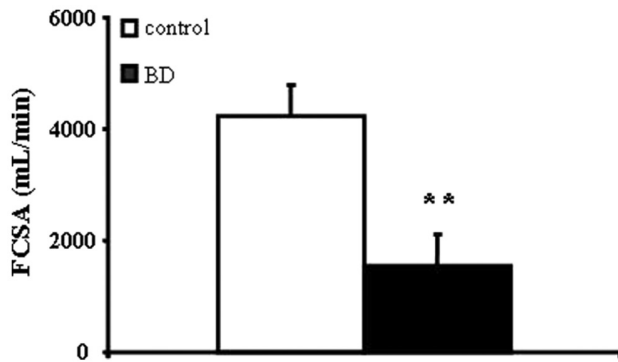


Figure 2: Functional capillary surface area (FCSA), a reflection of enzyme activity per vascular bed, is decreased in BD patients ($n = 8$) as compared to brain-injured controls ($n = 10$). Data are presented as means \pm SEM, $**P < 0.01$ from controls

concentrations in the capillaries, estimations of pulmonary endothelial ACE activity by the indicator-dilution technique is in practical terms equal to monitoring PCEB-ACE activity;^[9] it has been additionally shown that the contribution of plasma ACE to the single pass transpulmonary utilization of the substrate is minimal and that PCEB-ACE from alveolar capillaries with a diameter less than 20 μm are primarily responsible for the majority of the hydrolysis.^[26]

This method may additionally distinguish between abnormalities secondary to endothelial dysfunction per se (expressed by reduced $\%M$ and v) and decreased functional capillary surface area.^[15,17] PCEB-ACE activity reduction has been among the earliest signs of ALI in animal models, preceding acid-base imbalance, gas exchange and hemodynamic abnormalities, barrier dysfunction, and morphologic changes at the light and electron microscopy level.^[9] Assessment of PCEB-ACE activity in critically ill patients revealed that both transpulmonary hydrolysis and FCSA (alias A_{max}/K_m) decrease early during the ALI/ARDS continuum,^[16] while PCEB-ACE activity is also reduced in humans with pulmonary hypertension of diverse etiologies.^[14,17]

In the present study and in the absence of ALI or other apparent lung pathology, we detected significant reductions of both substrate $\%M$ and v in BD subjects as compared to brain-injured controls (Figures 1A and B). These data reflect reduced enzyme activity per capillary and may be related to (1) capillary endothelial dysfunction associated with alterations of either the PCEB-ACE microvascular concentration, the enzyme kinetic constants, or both (Equation 2); and (2) a decrease in capillary transit time (Equation 2). However, pulmonary capillary flow does not appear to change over a wide CO range,^[14,17,27,28] thus resulting in unchanged transit times. In addition, our BD patients had lower CO than the controls (Table 1) implying that, if anything, capillary flows would decrease resulting in higher capillary transit times and thus higher substrate

utilization as per Equation 2 (i.e., the opposite rather than the observed phenomenon). Thus, our BD patients exhibit true pulmonary capillary endothelial dysfunction, as compared to the brain-injured controls.

In addition, BD subjects exhibited significant decreases in FCSA as compared to brain-injured controls (Fig. 2). FCSA is proportional to the enzyme mass available for reaction (perfused capillary surface bed multiplied by the enzyme mass expressed on the endothelial surface) and the enzyme kinetic constants (Equation 3). Taken thus together, the observed decreases in substrate utilization and in FCSA, the latter should be related to both enzyme mass and/or kinetic constant reductions (capillary endothelial dysfunction), and to the lower CO and pulmonary plasma flows observed in the BD patients (Equation 3).

Could the decreased CO values observed in BD subjects, per se, cause the decreased enzyme activity parameters as compared to controls? As previously analyzed, this should indeed be partly the case for the lower FCSA observed in our BD subjects. We have, however, shown that substrate hydrolysis by PCEB-ACE in humans and animals does not change over a wide range of pulmonary blood flows.^[14,28] Capillary transit times could be increased under extremely low capillary flows,^[27] but such a phenomenon would have produced instead increased v and $\%M$. Thus a true, non pulmonary blood flow-related, reduction in PCEB-ACE activity appears to be present in our BD patients as compared to brain-injured controls.

An additional important issue is related to the potential systemic effects of low CO and if such effects could indirectly affect PCEB-ACE activity. In this respect, low CO is expected to lead to increased circulating natural ACE substrate angiotensin I through induction of the rennin angiotensin system (RAS). If such an increase could lead to pulmonary ACE saturation, the estimated PCEB-ACE activity would be expected to decrease. It has, however, been shown that circulating angiotensin I concentrations are significantly lower than the K_m of its interaction with ACE (~ 0.1 nM versus ~ 33 μM , respectively)^[29] meaning that PCEB-ACE saturation by angiotensin I is not possible. In a similar respect, an effect of the low CO via the inflammatory milieu cannot be excluded. It should be noted, however, that patients suffering from idiopathic pulmonary arterial hypertension exhibiting long-term low CO (lower than those observed in our BD group) did not show decreases in either BPAP $\%M$ or v compared with related controls despite the significantly higher CO of the latter,^[14] thus making such a possibility rather unlikely.

Several reports link systemic inflammation to pulmonary endothelial dysfunction.^[6] In an effort to investigate if the observed PCEB-ACE activity reductions coexist with

the presence of systemic inflammation, we additionally measured circulating inflammatory and brain injury-related biomarkers. A more prominent inflammatory process was detected in BD patients as compared to controls (Table 2); however, no significant relationship was observed among any inflammatory compound measured and the pulmonary endothelial ACE activity indices. Circulating CRP, PCT, and S100b in BD were all higher compared to controls (Table 2). Serum PCT levels have been found increased after BD, predicting early graft failure, especially in cardiac donors.^[30] Yet, a relationship of the above phenomenon to infection or systemic inflammatory changes in the brain-dead donor has not been established.^[31] The higher levels of the brain-specific marker S-100b observed in our BD patients reflect the presence and the severity of cerebral damage,^[32,33] but might also denote the major inflammatory impact of BD on peripheral organ dysfunction.^[34-36]

Most experimental and clinical studies confirm the presence of increased circulating proinflammatory cytokines shortly after BD occurs^[25,33,37-40] due to brain tissue ischemia itself and/or to α -adrenergic derangements.^[25,41] A massive inflammatory response is triggered following BD, characterized among others by increased serum levels of proinflammatory cytokines such as IL-6 and TNF- α as well as by upregulation of their receptors in peripheral organs; these phenomena appear associated with inferior donor organ viability.^[34,39,42-45] TNF- α and IL-6 participate in the early inflammatory response following BD by activating, among others, endothelial cells to express adhesion molecules (intercellular adhesion molecule-1 and E- and P-selectins);^[25] they additionally regulate the production of the potent neutrophil activator and chemoattractant IL-8 by endothelial and epithelial cells of the capillary alveolar membrane.^[6,36,46,47] Accordingly, our BD subjects exhibited increased plasma levels of both IL-6 and TNF- α (Table 2). However, despite the well-established effects of IL-6 and TNF- α on the pulmonary endothelium,^[8,9] our study does not provide direct evidence on a cause-effect phenomenon between these cytokines and the observed PCEB-ACE activity reduction in the BD patients.

Neutrophil infiltration in apparently healthy lungs of brain-dead donors has been shown to correlate with IL-8 levels in patients' bronchoalveolar lavage fluid (BALF), while elevated lung IL-8 expression was associated with graft failure post-transplantation.^[38] In contrast with the aforementioned seminal investigation, in our cohort lung and BALF IL-8 levels were not measured, not allowing for direct comparisons. In our study, no significant differences in plasma IL-8 levels were noted between BD patients and brain-injured controls, implying that this chemokine might not have contributed to the observed reductions seen in PCEB-ACE activity in the former.

In summary, this is the first study to demonstrate that subtle pulmonary endothelial dysfunction, as assessed by PCEB-ACE activity reduction, is present in BD patients in the absence of ALI or other overt lung pathology. Assessing pulmonary endothelial ACE activity at the bedside by means of indicator-dilution type techniques provides a direct and quantifiable index of pulmonary endothelial dysfunction that may reveal the existence of preclinical lung pathology in potential BD lung donors. Future studies should investigate the impact of such subtle pulmonary endothelial dysfunction in the outcome of lung transplantation.

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