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Severe pulmonary embolism decreases plasma L-arginine

To the Editor:

Measurements from humans and animal models with pulmonary embolism (PE) have demonstrated increases in multiple vasoconstrictive molecules, including prostaglandins, platelet-activating factor and leukotrienes [1]. Nitric oxide (NO), produced tonically by vascular endothelial nitric oxide synthase (eNOS), plays a pivotal role in maintaining a normal pulmonary vascular resistance under these conditions [2]. These facts underlie the construct hypothesis that in different subjects, PE can obstruct the same degree of pulmonary vasculature but manifest widely different pulmonary arterial resistances.

Preliminary evidence from animal models and humans suggests that acute PE is associated with intravascular haemolysis, related to the severity of PE [3–8]. Intravascular haemolysis liberates haemoglobin and diffusible haem, both of which directly bind NO. Ruptured erythrocytes also release large amounts of the enzyme arginase-1, which cleaves the eNOS substrate L-arginine, producing urea and L-ornithine.

We hypothesised that patients with acute PE that causes significant tricuspid regurgitation (TR) will have acutely increased plasma concentrations of arginase-1, decreased L-arginine and increased asymmetric dimethylarginine (aDMA) compared with patients who have mild PE without TR as well as patients without PE.

This was a secondary analysis of a four-centre prospective study of diagnostic accuracy conducted in patients with suspected PE (www.clinicaltrials.gov identifier NCT00368836) [9]. The enrolment and diagnostic criteria have been described previously [9]. Acute PE was considered present if two independent board-certified radiologists interpreted a filling defect consistent with acute PE on computed tomographic pulmonary angiography. Echocardiography was performed using techniques as previously described, including pulse-wave Doppler interrogation of the tricuspid regurgitant jet velocity measured >2.7 $m \cdot s^{-1}$, corresponding to an estimated right ventricular systolic pressure of 40 mmHg.

L-arginine concentrations were assayed in thawed, citrate-anticoagulated plasma (0.11 mM) using high-performance liquid chromatography (HPLC). L-Arginine standards (Sigma, St Louis, MO, USA) and unknown samples were precipitated with an equal volume of 10% perchloric acid and the neutralised supernatants were mixed with sodium tetraborate, L-norleucine, sodium cyanide and naphthalene 2,3-dicarboxaldehyde (dissolved in HPLC-grade acetonitrile), and 100-µL aliquots were resolved in a 30–60% acetonitrile gradient mobile phase on an octadecylsilane column using a Waters 616 LC System (Waters Corporation, Milford, MA, USA.) L-Arginine peaks were identified by location of external and internal arginine standards, and peak areas were corrected for quench of plasma and for stability of the derivatised L-arginine peak by the area of the internal standard L-norleucine peak. Concentrations were determined from a standard curve for L-arginine using the Empower software package (Waters Corporation). Plasma arginase-1 and haemoglobin protein concentrations were measured using commercially available ELISA assays (Human Arginase-1 I ELISA kit (Hycult Biotech, Plymouth Meeting, PA, USA) and Human Hemoglobin ELISA kit (Bethyl Laboratories, Montgomery, TX, USA)). Plasma carbonic anhydrase-1 (CA-1) was used as an additional biomarker of haemolysis and was measured with a custom sandwich ELISA. Briefly, monoclonal mouse anti-CA-1 capture antibodies (Abcam, Inc., Cambridge, UK) were immobilised on ELISA plate well bottoms (Nunc, Loughborough, UK) and incubated with 100 µL plasma sample, washed, and incubated with biotinylated goat polyclonal anti-CA-1 antibody and streptavidin conjugated to horseradish peroxidase as a chromogenic tag (wavelength 450 nm). Standard curves were constructed using human CA-1 (Sigma). Serum troponin I concentrations were measured with the iSTAT point-of-care system (Abbott Point of Care Inc., Princeton, NJ, USA) with an abnormal value considered to be >0.07 $ng \cdot mL^{-1}$.

Total dimethyl arginine content was assessed using tandem HPLC–mass spectrometry (MS) with heavy arginine as a standard and assuming a 1:1 racemic balance for asymmetric and symmetric methyl arginine enantiomers. Solid-phase extraction of plasma samples with a heavy aDMA internal standard were performed using an Oasis MCX cartridge (Waters Corporation). Components were separated by ultra-performance liquid chromatography (NanoACQUITY UPLC; Waters Corporation) and quantified using gas chromatography–MS/MS (TSQ Quantum XLS; Thermo Scientific, Waltham, MA, USA).

Data were tested for normality (Shapiro–Wilk $p<0.1$). Normally distributed data were compared with a one-way ANOVA with multiple comparisons using Tukey's *post hoc* test. Medians were compared using the Kruskal–Wallis test with pairwise comparisons using the Cononver–Inman test. $p<0.05$ was considered significant (StatsDirect version 2.6.2; StatsDirect Ltd, Altrincham, UK).

We enrolled 109 patients with PE including 44 with PE causing a mean ± SD pulmonary vascular obstruction of 33 ± 28%. Doppler assessment found an abnormally high tricuspid regurgitant jet velocity (>2.7 $m \cdot s^{-1}$) in 20 patients (PE^+ TR^+). Patients with PE were similar terms of age and comorbidities compared with patients who did not have PE. The troponin I concentration, which evidences increased PE severity, was elevated in 12 (60%) out of 20 PE^+ TR^+ patients, two (8%) out of 24 PE^+ TR^- patients and in two (3%) out of 65 PE^- patients.

PE^+ TR^+ patients had higher median (interquartile range) free plasma carbonic anhydrase-1 (70.5 (37.2–13.6) $\mu g \cdot L^{-1}$) than PE^- patients (50 (23–137) $\mu g \cdot L^{-1}$). PE^+ TR^- patients had elevated haptoglobin (2124 (1466–2621) $mg \cdot L^{-1}$), reflecting an acute-phase increase in the setting of acute thrombosis, and haptoglobin was significantly lower in PE^+ TR^+ patients (580 (468–678) $mg \cdot L^{-1}$) than PE^+ TR^- patients, but was not significantly lower than in controls (888 (642–1362) $mg \cdot L^{-1}$). The plasma-free haemoglobin concentrations were not significantly different between groups, suggesting effective haptoglobin scavenging.

PE^+ TR^+ patients had higher arginase-1 and aDMA, and lower L-arginine values than patients without PE ($p<0.05$ by ANOVA and Tukey's *post hoc* test) (fig. 1).

This study documents the decreased blood L-arginine concentrations in patients with PE that was severe enough to cause a tricuspid regurgitant jet velocity >2.7 $m \cdot s^{-1}$. These patients also had increased free plasma concentrations of the erythrocyte enzymes CA-1 and arginase-1, and increased dimethylarginine, together with decreased L-arginine compared with control patients who had no PE. Patients with PE and minimal or no TR had values of these biomarkers similar control patients without PE. Cases (PE^+) and

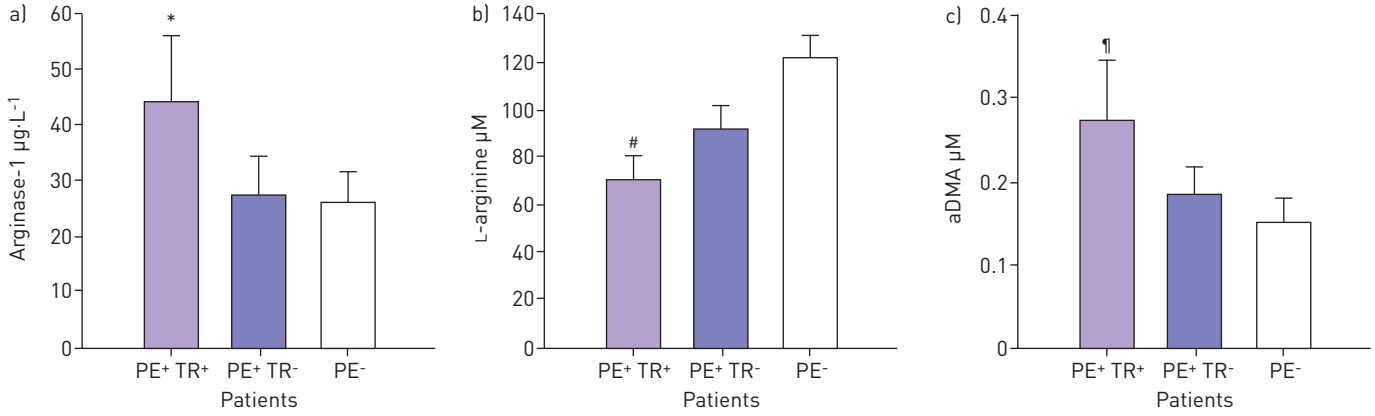

FIGURE 1 Plasma concentrations of a) arginase-1, b) L-arginine and c) asymmetric dimethylarginine (aDMA). PE: pulmonary embolism; TR: tricuspid regurgitation *: $p<0.05$ *versus* PE^+ TR^- and PE^- patients using Tukey's *post hoc* test; #: $p=0.03$ *versus* PE^- patients; ¶: $p=0.04$ *versus* PE^- patients.

controls (PE^-) were well matched in terms of age and comorbidities. These data provide evidence that acute PE with TR is associated with haemolysis, depression in the substrate for eNOS and increase in an inhibitor of eNOS.

The hypothesised timing and location of shear upon erythrocytes with acute PE would require release of only a small amount of free haemoglobin to cause clinically important pulmonary vasoconstriction. The pulmonary vasoconstrictive effect of haemolysate has been well documented in isolated lung preparations [3, 10]. Upon its rupture, the erythrocyte releases tetrameric ($\alpha_2\beta_2$) haemoglobin, which can immediately and avidly bind NO, but haemoglobin must first dissociate into $\alpha\beta$ dimers before haptoglobin can bind and inactivate this NO scavenging effect, an effect that may require a few seconds to occur [11]. In contrast to the millimolar concentrations required to constrict peripheral vasculature, free haemoglobin in the low micromolar concentration range will significantly increase pulmonary vascular resistance by NO scavenging [5, 12].

In conclusion, this report presents the first published data from humans to show decreased plasma L-arginine in patients with more severe PE, together with evidence of haemolysis. These data are consistent with the hypothesis that intravascular haemolysis contributes to pulmonary hypertension in patients with acute PE.

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More severe PE reduced L-arginine and increased dimethylarginine concentrations, which could reduce NO biosynthesis http://ow.ly/qMAeT

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Prediction of outcome after PEA in chronic thromboembolic pulmonary hypertension using indexed pulmonary artery diameter

To the Editor:

Chronic thromboembolic pulmonary hypertension (CTEPH) is associated with considerable morbidity and mortality [1]. It occurs in 2–4% of patients after acute pulmonary embolism [2]. Pulmonary endarterectomy (PEA) is the treatment of choice to relieve pulmonary artery obstruction in patients with CTEPH and has been remarkably successful [3]. However, in 10–50% of patients PEA is not possible due to either distal pulmonary vascular obstruction that is surgically inaccessible or significant comorbidities thought to be associated with an unacceptably high risk [4]. Therefore, careful selection of operable candidates is paramount. A correlation between the main pulmonary artery (PA) diameter and pulmonary haemodynamic parameters before PEA have been described in CTEPH patients [5]. We evaluated whether preoperative PA diameter indices could predict the occurrence of mortality and clinical worsening after PEA.

A multidisciplinary panel including pulmonologists, radiologists, cardiologists, and cardiothoracic surgeons reviewed each case. Patients were considered suitable for surgery when they were symptomatic, had an elevated pulmonary vascular resistance (PVR) (>250 dyn·s·cm^{-5}), segmental or more proximal lesions and no severe comorbidity. Before surgery all patients underwent standardised work-up as described earlier [4]. The study was approved by the local ethical committees of University Hospitals Leuven, Leuven and St Antonius Hospital, Nieuwegein.

According to the study by HEINRICH *et al.* [5], the widest diameters of the ascending aorta (Ao) and the widest diameter of the main PA perpendicular to its long axis were measured at the level of the bifurcation of the PA. The ratio of the PA and the Ao diameters was calculated [5]. The PA diameter was indexed by body surface area (BSA).

Clinical worsening was defined as the combination of death, need for pulmonary arterial hypertension (PAH) medication initiated after PEA in the presence of persistent or residual pulmonary hypertension (PH) or a 15% decrease in 6-min walk distance (6MWD) in comparison with the best postoperative value, without improvement in New York Heart Association (NYHA) functional class during follow-up. Clinical worsening was determined by the event which was reached first.

Persistent or residual PH after PEA was defined as mean pulmonary artery pressure (PAP) >25 mmHg by right heart catheterisation or systolic PAP >40 mmHg by echocardiography [4].