Coronavirus disease 2019 may be driven by an overactivation of the renin-angiotensin-aldosterone system

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Abstract

SARS-CoV-2 enters the cell through the ACE2 receptor, which is considered one of the main inhibitors in the Renin-Angiotensin-Aldosterone System (RAAS).^{1,2} The virus has been shown to downregulate the ACE2 receptor, leading to a subsequent increase in the vasopressor angiotensin II.³ Evidently, critical coronavirus disease 2019 (COVID-19) is thought to be due to a dysregulated immune response, causing a cytokine-release syndrome eventually leading to acute respiratory distress syndrome (ARDS).^{4,5} However, several reports on clinical laboratory features and case-descriptions of critically ill patients with COVID-19 show discrepancies compared to typical ARDS. Here, we show that infusing swines with angiotensin II induces a pathophysiological syndrome closely resembling that of patients with RT-PCR-positive COVID-19. By using multimodal clinical imaging of patients, comparing laboratory data and translational histological features, we show that it is highly likely that an increase in RAAS is one, if not the main, pathogenic feature in critical COVID-19. Furthermore, it is plausible that this large animal model can be used to screen for potential new treatments for patients with severe COVID-19 and that MRI lung perfusion can be used to evaluate the outcome of potential treatments targeting the pathophysiological syndrome.

Introduction

SARS-CoV-1 and SARS-CoV-2 both enter human cells through cell surface angiotensin-converting enzyme 2 (ACE2) receptors.¹ SARS-CoV-1 downregulates ACE2,³ with a subsequent increase in angiotensin II (ANGII) levels,⁶ which potentially creates a disruption akin to overactivating the Renin-Angiotensin-Aldosterone System (RAAS).² Current clinical management guidelines for the coronavirus disease 2019 (COVID-19) are mainly centred around the assumption that SARS-CoV-2 directly results in an acute lung parenchymal disease such as acute respiratory distress syndrome (ARDS).^{4,5} The possible underlying mechanisms are thought to be viral ARDS and/or high levels of cytokines leading to a cytokine-release syndrome.⁴ However, the initial clinical presentation of COVID-19 in the intensive care units (ICU) at our hospital, as well as described by others, does not follow a typical ARDS-pattern.⁵ Further, the levels of pro-inflammatory cytokines can be lower than the levels that are expected during cytokine-release syndrome.⁷ Moreover, a case series from Wuhan described that ANGI levels were markedly elevated and linearly associated with viral load and lung injury.⁸ We found that infusion of ANGI in three sedated swine within hours induced a clinical syndrome similar to that observed in ICU patients in COVID-19, with histological changes in the lungs after only five hours with severe thickening of the alveolar walls, possible hyaline membranes and clotting of vessels, as previously reported in humans in COVID-19.^{9–11} We present data in reverse-transcriptase–polymerase-chain-reaction (RT-PCR) confirmed COVID-19, showing high pulmonary artery (PA) pressures in one patient and extensive peripheral hypoperfusion of the lungs using magnetic resonance imaging (MRI) in another patient. We also present computed tomography pulmonary angiography (CTPA) results from 289 consecutive SARS-CoV-2 RT-PCR-positive patients and a total of 339 CTPA examinations; 17% of scans revealed pulmonary thrombosis/embolism and 60% of the scans had a wide PA diameter, suggestive of pulmonary hypertension, further supported by echocardiography findings in a sub-cohort of 50 patients. We also relate our findings to the results observed in swines: ANGI infusion leads to high PA pressures and a marked reduction in bleeding time. D-dimer increases were observed, as seen in COVID-19 patients. Paradoxically, fibrinogen increased, suggesting a possible link between ANGI infusion and fibrinogen synthesis. All three swines showed pulmonary thrombotic material without deep venous thrombosis, suggestive of local thrombotic events. We suggest that much of the clinical presentation in ICU patients with COVID-19 could be driven by a loss of the inhibition of the RAAS, causing supranormal concentrations of ANGI. Based on these results, we believe that critical COVID-19 is primarily a vascular and coagulation disease that is driven by a viral disruption of the RAAS and that focus should, henceforth, be directed towards its understanding and therapeutic targeting.

Results

Two clinical cases

We performed lung perfusion MRI in a SARS-CoV-2 RT-PCR-positive 61-year-old male on ICU day 22. Initial presentation to the ICU included a PaO₂ of 7.6 kPa and PaCO₂ of 3.6 kPa by arterial blood gas; serum measurements of IL-6 154 ng/L, TNF- α 10.2 ng/L, C-reactive protein 242 mg/L, ASAT 1.08 μ kat/L and D-dimer 0.94 mg/L FEU. We show that the maximum contrast bolus concentration in the PA comes at 15 seconds after injection and that the maximum contrast bolus concentration reaches the aorta after 20 seconds (Fig. 1). Using these values, we calculated a time-to-peak (TTP) map of the lungs using a colour lookup table where blue and red corresponds to 14 and 21 seconds respectively. All red parts of the lung have a maximum contrast bolus concentration later than the aorta (sic). To further understand these paradoxical values, we measured regions of interest in the lungs without infiltrates, as identified by T2-weighted anatomical images. The most probable conclusion is that peripheral regions of the lungs do not receive a contrast bolus up to 10 seconds after the contrast has peaked in the aorta. In conclusion, using MRI perfusion, we demonstrate a lack of contrast bolus enhancement in the peripheral lung parenchyma. In the second patient, a SARS-CoV-2 RT-PCR-positive 58-year-old male, we placed a PA-catheter on ICU day 25 and recorded systolic and diastolic PA pressures of $51 \pm 2.9 / 11 \pm 3.2$ mmHg (mean $\pm$ SD) and SvO₂ of 62.4 ± 1.3 % (mean $\pm$ SD), both during the first hour after placing the catheter. Upon admission to the ICU, the patient's clinical chemistry values were PaO₂ of 8.0 kPa and PaCO₂ of 5.1 kPa, IL-6 at 95 ng/L, TNF- α at 9.8 ng/L, C-reactive protein at 66 mg/L, ASAT 0.53 μ kat/L, D-dimer of 0.26 mg/L FEU.

Retrospective imaging cohort

Computed tomography pulmonary angiography

To further confirm our findings, we retrospectively analysed all consecutive CTPA examinations acquired at Karolinska University Hospital in Huddinge, Stockholm, Sweden, in patients with RT-PCR-confirmed COVID-19. In total, 339 examinations were performed in 289 adult patients with RT-PCR-confirmed COVID-19. Motion artifacts or other technical issues prohibited PA-diameter measurements in 47 examinations, as measured by the gold standard reader TG, resulting in 292 analysed scans. The inter-rater agreement was excellent (N=290, ICC=0.97, $P<0.001$). The mean PA-diameter was 29.2 ± 4.3 mm (mean $\pm$ SD), with 174 of 292 (60%) of examinations with COVID-19 demonstrating a PA diameter ≥ 28 mm, suggestive of pulmonary hypertension.¹² Clinical readings in the same cohort revealed that 56 of 336 examinations (17%, 3 scans were not interpretable) revealed pulmonary thrombosis/embolism.

Echocardiography

All echocardiography measurements indicated high PA pressure: maximal tricuspid regurgitation velocity (TR-V_{max}) 3.0 ± 0.43 (N=38; normal reference ≤ 2.8) m/s; estimated systolic pulmonary artery pressure (SPAP) 47 ± 14 (N=38; normal reference < 36) mmHg; right ventricular outflow tract acceleration time (RVOT-AT) 57 ± 43 (N=17; normal reference > 100) ms.^{13,14} TR-V and SPAP were not measurable in 12 patients due to insufficient tricuspid valve regurgitation doppler signal. In 4 patients, only the central venous pressure could be estimated but is not reported since many patients were intubated. In 2 patients, none of the parameters were obtainable due to insufficient technical image quality.

Large animal physiology

To further understand our clinical findings, we explored the systemic effects of the RAAS by infusing three sedated swines with ANGII. Within five minutes, arterial and PA blood pressures started to climb (Fig. 2). Target arterial systolic blood pressures were set at 150 mmHg and the start dose of ANGII was adopted from the ATHOSIII trial.¹⁵ Swine #1 was subject to 530 minutes of ICU care and reached PA-systolic (PAs) pressures exceeding 30 mmHg after 360 minutes. Swine #2 reached PAs > 30 mmHg after 30 minutes and died of acute right ventricular heart failure with extensive pulmonary thrombosis/embolism after 300 minutes. Swine #3 reached PAs > 30 mmHg in 390 minutes. ANGII infusion further induced a rapid decline in SvO₂, falling below 50% in all swines. Swine #1 had a warm body temperature, which is a paradoxical finding when administering a vasopressor. Surface body temperature was measured in Swine #2 and #3 and skin temperature did not differ more than 2 degrees from 90 minutes into the experiment until death. Arterial blood gas sampling showed declining trends in PaO₂ and trends towards a small increase in PaCO₂ (Fig. 2). No lactate could be detected in the blood.

Large animal radiology and dissection

When performing an autopsy on Swine #1, we found a 13 cm long pulmonary thrombus (Fig 3c). To exclude embolisation, we performed ultrasonography of the hind legs to screen for deep venous thrombosis (DVT) every second hour in Swine #2 and #3, but none were detected. However, we did find extensive pulmonary thrombosis formation in Swine #2 visualised by conventional angiography and later confirmed at autopsy (Fig. 3). Swine #3 had disseminated thrombotic material in the pulmonary arteries, as illustrated by a supplementary dissection videos. Ultrasonography without DVT of the hindlimb suggests local clot formation in the pulmonary circulation and not embolic material from the deep venous system. Macroscopically, we found wedge-shaped areas of red discoloration in the periphery of the lung (Fig. 3a). Samples for histology were acquired from these areas.

Large animal histology

The lung sample unexpectedly sank to the bottom of the formaldehyde solution (i.e. not floating as expected from healthy lung tissue). A red discoloration in the frozen sections was noted in the cryostat and the lung was surprisingly easy to cut, without the normal tendency of shattering when cutting snap-frozen lung tissue. After hematoxylin and eosin staining, we observed thrombotic material in both large and small vessels of the lungs. We found severe thickening of the alveolar walls and what might be hyaline membranes and/or debris in the alveolar sacs (Fig 3).

Large animal clinical chemistry data

We performed bleeding time assessments in Swine #2 and #3 with baseline bleeding times of 285 seconds and 255 seconds respectively. These were reduced to < 120 seconds after 90 minutes and remained low for the remainder of the experiment. D-dimer increased in Swine #1, doubling after 120 minutes, and almost doubled in Swine #2 after 120 minutes. Paradoxically, fibrinogen also increased or remained unchanged in spite of increased D-dimer, suggesting a possible unknown link between ANGII and fibrinogen synthesis, not caused by an acute phase reaction, since IL-6 and TNF- α remained below the level of detection in all swines during the experiment. Osmolality successively increased in all three swines by > 11 mosmol/kg through another unclear mechanism. We measured no changes from baseline values of Owrens PT (INR), APT-time, antithrombin, C-reactive protein, ferritin, albumin, creatinine, cystatin C, urea, ASAT, ALAT, γ -GT, LD, CK, myoglobin or triglycerides.

Discussion

Our results demonstrate a similar pattern between patients with COVID-19 admitted to the ICU and ANGII-infused swine. ANGII has previously been used in the ATHOSIII clinical trial.¹⁵ Sub-analyses of this trial highlighted important

thrombotic and infectious complications associated with ANGII.¹⁶ If SARS-CoV-1 disturbs the distribution of the ACE2-receptor to produce a subsequent increase in ANGII,³ it is likely that the same mechanism of receptor disruption in SARS-CoV-2 leads to the same effects. Further, a linear relationship seems to exist between ANGII levels and clinical outcome.⁷ Infusion of ANGII, resulting in an acceleration of the RAAS, induces the same clinical presentation as a loss of RAAS brake, or a loss of ACE2-receptors. The most appropriate dose of ANGII used in swines can be debated, however, injection of an ACE2 blocker (PD123319) in humans leads to an increase in mean arterial pressure of 12 mmHg after five minutes.¹⁷

In the pulmonary circulation, using lung perfusion MRI, we demonstrated a reduction and, in some areas, likely cessation of blood flow, even in the absence of infiltrates. We further support these findings by CTPA and echocardiography in a retrospective patient cohort. There are a number of methods to evaluate possible signs of increased pressure in the lung vasculature bed using CTPA. One of the most commonly applied methods in a clinical setting is measuring the PA diameter. Axial PA diameter measurements have been found to be a relatively sensitive parameter for detecting even borderline pulmonary hypertension with a sensitivity of 80% and a specificity of 62%, when using a cutoff ≥ 28 mm.¹² Given the characteristics of the measurement, it is thus likely that our measurements somewhat underestimate the fraction of patients with increased PA pressure, which is supported by our echocardiography findings indicative of elevated PA pressures. In combination, our findings provide multimodal evidence of an elevated PA pressure as a hallmark of COVID-19 and multiple lines of evidence in support of our two case reports and further our swine model data.

We believe our results indicate that COVID-19 patients are prothrombotic, as observed in the ICU, and that this may be caused by disruptions in at least two tenets of Virchow's triad, including: stasis in the pulmonary blood flow by vasoconstriction and a hypercoagulable state with increased fibrinogen synthesis, shortened bleeding times and rather distinct histological changes after only 500 minutes of ANGII infusion in our swine models. Other publications even suggest disruption of the third tenet of the triad, by endothelial dysfunction due to endothelitis.¹⁸ Altogether, these findings support our conclusion that severe COVID-19 is a vascular syndrome with a hypercoagulable state. Our results are translations between a large animal model and the clinical presentation of patients with COVID-19 in the ICU, therefore, these findings may only be considered preliminary. The CTPA cohort is retrospective and lacks an internal control group. However, all the cumulative agreement of the individual findings lends support to our hypothesis. Furthermore, the hypothesis of viral-ARDS or cytokine-release syndrome should probably be considered weakened by the observed pathophysiological effects in both large animals and clinical data.

If our assumptions are true, then many of the currently ongoing clinical trials may prove less successful than symptomatic targeting of vascular/coagulation disturbances or dysregulation of the RAAS. Our retrospective cohort of CTPA demonstrates a high incidence of pulmonary embolism in COVID-19 patients. Extended use of CTPA and treatment with anticoagulation therapy is therefore warranted in clinical guidelines. In a time of pandemic disease, we urge capable large animal laboratories to obtain ethical permissions to validate our findings and encourage clinical centres with MRI capabilities to evaluate the potential clinical benefits of lung perfusion MRI. We will assist any clinical site by answering questions about MRI protocols to the best of our abilities. We will continue to model severe COVID-19 as a vascular syndrome to advance our understanding of the pathophysiological mechanisms and use this model for large scale pre-clinical therapy evaluation. We will also use lung perfusion MRI to evaluate our patients when translating these preclinical findings to our ICU patients.

Methods

Magnetic resonance perfusion

This case is submitted with written consent by next of kin. A 1.5 Tesla Philips Ingenia MRI scanner, software version R 5.4 (Philips Healthcare, Best, Netherlands), was used for perfusion analysis. An anatomical T2-weighted sequence was used to identify lung infiltrates with the following parameters: MultiVane, 2D field-of-view 460×460 mm, voxel size 0.9×0.9×4 mm, echo/repetition times at 100/2458 ms with a compressed sensing-sensitivity encoding factor 2, 1 average and 36 slices. For the dynamic contrast series, we used 4D time-resolved MRI angiography with a keyhole T1-weighted gradient-recalled-echo with field-of-view 500×500 mm, voxel size 1.5×1.5×4 mm, echo/repetition times at 1.8/3.78 ms, a compressed sensing-sensitivity encoding factor 3.6, 1 average with 16 dynamic phases and 60 slices. A Max 3 contrast injector (Ulrich Medical, Ulm, Germany) was used to administer gadolinium-based contrast agent: gadobutrol solution, 1.0 mmol/ml, 2 ml, followed by 20 ml 0.9% saline solution with 5 ml/s. The first phase acquired the entire K-space in 8.3 s and subsequent phases used a keyhole acceleration with 20% scanning, resulting in a temporal resolution of 1.7 s per phase. TTP maps were generated using the T1 MRI perfusion application in Philips Intellispace v10.1.3 (Philips Healthcare, Best, Netherlands).

Computed tomography pulmonary angiography

A consecutive cohort of all patients with RT-PCR-confirmed COVID-19 undergoing CTPA at Karolinska University Hospital in Huddinge, Stockholm, Sweden, between March 2nd (first patient admitted) and May 20th were retrospectively and independently evaluated by two raters (T.G., radiologist; J.A., 4th-year medical student) according to a standardised scheme,¹⁹ after consultation of a senior thoracic radiologist (M.K.). The ratings of the radiologist was considered the gold standard and the inter-rater agreement was assessed by intraclass correlation coefficient. All CTPA imaging was performed on Siemens SOMATOM Definition Flash (Siemens Healthineers, Erlangen, Germany), GE Discovery CT750 HD and Revolution CT (GE Healthcare, Milwaukee, USA). A flow chart describing the CTPA cohort can be found in Fig. 4.

Echocardiography

Echocardiography data was retrospectively analysed in a sub-sample (N=50) of patients undergoing CTPA imaging by a senior clinical physiologist (G.A.) to extract the TR-Vmax, SPAP and RVOT-AT, as previously described, with normative values from the literature.^{13,14} All echocardiography was performed on GE Vivid S70 (GE Healthcare, Milwaukee, USA). The CTPA and echocardiography sub-study was approved by the Swedish Ethical Review Authority (no. 2020-01895); Informed consent was waived due to the retrospective nature of the study.

Clinical and preclinical measurement of PA pressure

This case is submitted with written consent by next of kin. A Swan-Ganz catheter (Edwards Lifesciences, Irvine, USA) for measurements of PA pressure was placed in the reported patient and in all of the swines. In the patient, it was placed from the jugular vein and in the swines from the femoral vein and connected to a HemoSphere and Vigilance monitor (Edwards Lifesciences, Irvine, USA) respectively.

Swine experimental setup

This study took place at the Karolinska Experimental Research and Imaging Centre, Karolinska University Hospital, Stockholm, between May 11th and May 15th, 2020. All animal studies were conducted according to Karolinska Institutet guidelines for animal experiments. The study was approved by the Regional Ethics Committee for Animal Research in Stockholm, Sweden (no. 6716-2020). Three swines with weights between 36 and 39 kg were used in this study. Each animal fasted for 12 hours with free access to water before the procedure. They arrived sedated after premedication with intramuscular ceptor vet 1 mg/ml-zoletil 100 (Vetmedic/Virbac, Thirsk, UK) 0.8–1 mg/kg.

Induction of anaesthesia was conducted with pentobarbital (Sandoz, Holzkirchen, Germany) 1–3 mg/kg and fentanyl (B. Braun, Melsungen, Germany) 2.5 µg/kg as an intravenous bolus dose. Maintenance of anesthesia was achieved with continuous infusion of pentobarbital (0.1–0.2 mg/kg/min) and morphine (Meda, Solna, Sweden) (0.1–0.25 mg/kg/h) titrated to a moderate depth of anesthesia. No muscle relaxants were used during the experiment.

Intubation using an endotracheal tube (7.0) was performed after induction of anesthesia and during spontaneous breathing. The animals were normoventilated by pressure-controlled ventilation with Siemens Servoventilator 900C (Siemens Healthineers, Erlangen, Germany) with a total tidal volume of 10 mL/kg and with an inspiratory oxygen fraction (FiO₂) of 0.21. End-tidal CO₂ was monitored with a capnography incorporated in the surveillance system (Datex, GE Healthcare, Milwaukee, USA). Ventilation and respiratory frequency were adjusted to maintain end-tidal CO₂ between 4.7–5.3 kPa. A 5-lead electrocardiogram was recorded continuously as well as arterial pulse oximetry (SpO₂) for peripheral oxygen saturation (Datex, GE Healthcare, Milwaukee, USA). The Pulse oximetry was placed on the tail. All animals received a urine catheter and hourly diuresis was recorded. Prior to skin incision, local anesthesia (lidocaine 10 mg/ml, Aspen Nordic, Ballerup, Denmark) <0.4 mg/kg) were given. A 7 F central venous catheter (Terumo, Tokyo, Japan) was inserted by open technique via the left internal jugular vein for administration of drugs. ANGII infusion started at 20 ng/kg/min in Swine #1 and were stepwise elevated to 80 ng/kg/min in 60 minutes, followed by a stepwise increase to a maximum dose of 240 ng/kg/min after 515 minutes except for Swine #2 who suffered acute right ventricular heart failure at a dose of 100 ng/kg/min and died. This schedule was then used in subsequent swines. Bleeding time was assessed by cutting the skin of the ear for 10-15 mm and removing any blood every 15 seconds. When no further bleeding could be identified, this was determined to be the bleeding time.

Preclinical imaging

All conventional angiography was performed with a Philips XD20 angiographic system and 3DRA workstation (Philips Healthcare, Best, Netherlands). Visipaque 270 contrast agent (GE Healthcare, Milwaukee, USA) was used for all contrast-enhanced applications. Xper-CT images obtained were reviewed using the XD20 systems XperCT high dose program and soft tissue algorithms.

For endovascular access, we punctured the vessels using a micropuncture set (Merit Medical AB, Stockholm, Sweden) guided by ultrasonography with Siemens Acuson Sequoia 512 (Siemens Healthineers, Erlangen, Germany). Access was established with an introducer in the right femoral artery, the right femoral vein (Swine #1 and #3) and the left femoral artery (Swine #2). We used a 7 French introducer except for the PA catheter where we used an 8 F introducer (Terumo, Tokyo, Japan). We placed the 7.5 F PA catheter (Edwards Lifesciences, Irvine, USA) by fluoroscopy guidance and the location was confirmed by invasive pressure. A distal access guide (Envoy 6 F, Cordis, Santa Clara, USA) was then placed in the proximal part of the descending aorta for continuous monitoring of aortic pressure and a 7 F pigtail catheter was placed in the right atrium of Swine #2 for pulmonary angiography. Ultrasonography was performed every second hour during the experiment to exclude deep venous thrombosis in the hind legs.

Pre-clinical chemistry analysis

Blood samples were acquired at baseline and at 120, 240, 360, 435, 545, 665, 760 minutes or directly prior to death when systolic blood pressure dropped below 70 mmHg. Arterial blood gases were obtained at baseline, 75, 270, 345, 435, 545, 665, 750 min or when close to circulatory collapse. Citrated platelet-poor plasma and serum samples were analysed using proprietary assays at the Karolinska University Laboratory, accredited according to ISO 15189 by the Swedish Board for Accreditation and Conformity Assessment. Coagulation parameters were analysed on the Sysmex

CS-5100 System (Siemens Healthineers, Erlangen, Germany), chemistry analyses were analysed on the Cobas 6000 Analyzer (Roche Diagnostics, Basel, Switzerland) and osmolality in serum was analysed with the Osmometer Advanced 2020 Multi-Sample (Advanced Instruments, Norwood, USA).

Pre-clinical histological analysis

Lungs samples were placed in 4% formaldehyde at +8°C for 24 hours. The formaldehyde was then exchanged. Tissue samples were then snap-frozen in isopentane containing dry ice and cut using a Leica CM 3050 S cryostat (Leica, Wetzlar, Germany). Samples were collected on Superfrost plus glas (Thermo Fisher Scientific, Waltham, USA) and Mayer's Hematoxylin and Eosin 0.2% (Histolab AB, Stockholm, Sweden). Glasses were mounted with glycerol:PBS with cover glasses (VWR International, Radnor, USA), put in a Zeiss Axio Scope.A1 (Carl Zeiss AG, Oberkochen, Germany) and pictures were acquired by a Samsung Galaxy S10 Plus (Samsung, Seoul, South Korea) through the microscope oculars.

Statistical analysis

Statistical analyses were performed using MATLAB (version R2019b, The Mathworks Inc., Natick, USA) and IBM SPSS Statistics version 25 for Mac (IBM, Armonk, USA). A threshold of $P < 0.05$ was considered to be statistically significant. Statistical analysis of manually drawn regions of interest in normal-appearing lung tissue on MRI perfusion were analysed using MATLAB. Extrapulmonary and extramediastinal tissues were manually masked. Other descriptive statistics on MRI perfusion were also generated in MATLAB. Inter-rater agreement of CTPA measurements were calculated using intraclass correlation coefficient of average measurements in SPSS.

Data availability

The authors will freely make available any materials and information associated with their publication that are reasonably requested by others for the purpose of academic, non-commercial research.

Declarations

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Competing interest: The authors declare no competing interest.

Contributions

SR, MJF and JL designed the animal studies and together with JA performed the animal studies and analysed data. JA, RO, TG and JL performed pre-clinical histological evaluation. SR, MF, MJF and JL analysed clinical chemistry

data. SR and FCJ stressed the need for adequate imaging in COVID-19 and took care of the ICU patient during the MRI. RVP and JL designed the MRI protocol. SN and JL interpreted the MRI study. JA, MK, RO, TG and JL analysed computed tomography pulmonary angiography data. GA performed and analysed echocardiography data. SR and FCJ performed the clinical pulmonary artery catheter measurements and analysed the data. MJF and JL supervised the study. JL wrote the first draft and all authors revised the manuscript critically.

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Figures

Figure 1

MRI of a patient with COVID-19 shows a severe peripheral perfusion deficit. a, Anatomical coronal T2-weighted image used to ensure that the regions of interest were placed in lung tissue with normal-appearing T2 signal. b Coronal time-to-peak (TTP) map where the colour lookup table is set so that blue corresponds to the contrast TTP in the pulmonary artery and yellow the TTP in the aorta. c Time Intensity curves in a region of interest in the pulmonary artery (blue), aorta (red), a hilar region (yellow) and a peripheral region (green) (mean $\pm$ SD whisker bars). d, Rescaled signal intensity curves with the same hilar and peripheral region of interests as displayed in c. Yellow line represents a hilar region with a peak of contrast at 18.5 seconds post-infusion, e.g. during the expected time between the pulmonary and aortic peaks. Green line represents a ventilated peripheral region with normal-appearing T2 signal but without bolus passage (i.e. ventilation/perfusion-mismatch).

Figure 2

Arterial blood pressure and gas measurements in the large animal study. a, Mean systemic arterial pressure and pulmonary arterial pressure is illustrated with mean and whisker bars represent the standard deviation. Graphical data is truncated at 540 min and time = 0 represents the start of ANGII infusion. After the 540 min time point, only Swine #3 was still alive and, hence, a standard deviation cannot be calculated. b, Arterial blood gases with red line for PaO₂ and blue line for PaCO₂, also mean $\pm$ SD.

Figure 3

Macroscopic and microscopic findings in swine lungs after 500 minutes of angiotensin II infusion. a, Black arrows indicate wedge-shaped peripheral pathology within the inferior lobes in both lungs of a swine. b, The white arrow identifies a large pulmonary thrombus, c, example of a 13 cm long pulmonary thrombus. Histological imaging of the lung area affected by the pulmonary infarcts, d, e, f, revealed alveolar wall thickening and accumulation of cellular debris. The provided photomicrographs are at 20x magnification (d) and 40x magnification (e, f).

Figure 4

Flow chart of the CTPA cohort and subsequent analyses.

Supplementary Files

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