

performed at home by the kidney transplant recipients (KTR) themselves, followed by isolation of total RNA from the lysate and mRNA enrichment using a silica-membrane-based cartridge, both performed at the Core Laboratory. Using the HPP, total RNA was isolated from kidney allograft biopsy-matched urines and absolute copy numbers of CD3 $\beta$  mRNA, CXCL10 mRNA, and 18S rRNA, components of the Clinical Trials in Organ Transplantation 04 (CTOT-04) three-gene TCMR diagnostic signature, and urinary cell BKV VP1 mRNA copy number, were measured using customized RT-qPCR assays. RESULTS/ANTICIPATED RESULTS: CTOT-04 three-gene TCMR diagnostic signature scores in urine processed using HPP discriminated KTR with TCMR (12 TCMR biopsies from 11 KTR) from KTR with no TCMR/BKVN (29 No TCMR/No BKVN biopsies from 29 KTR) ( $P=0.0005$ , Mann-Whitney test), and AUROC was 0.84 (95% CI, 0.69 to 0.98). TCMR was diagnosed with sensitivity of 67% (95% CI, 35 to 89) at a specificity of 86% (95% CI, 67 to 95) using the CTOT-04 validated cutpoint of  $-1.213$  ( $P=0.0016$ , Fisher exact test). BKV VP1 mRNA copy number in urine processed with HPP discriminated KTR with BKVN ( $n=7$ ) from KTR with no TCMR/BKVN ( $n=29$ ) and AUROC was 1.0 (95% CI, 1.00 to 1.00). BKVN was diagnosed with a sensitivity of 86% (95% CI, 42 to 99) at specificity of 100% (95% CI, 85 to 100) with the previously validated cutpoint of  $6.5 \times 10^8$  BKV VP1 mRNA copies/ $\mu\text{g}$  of RNA (P DISCUSSION/SIGNIFICANCE: Urine processed using the HPP predicted TCMR and BKVN in KTR. The HPP represents not only a significant advance towards portability of urinary cell mRNA profiling but also should improve patient management by minimizing visits for urine collection.

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### Oxygenated Peritoneal Perfluorodecalin Improves Response to Normobaric Hypoxic Exposure in Swine

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OBJECTIVES/GOALS: Patients suffering from respiratory failure have few options to support oxygenation and carbon dioxide removal aside from mechanical ventilation. Our objective was to test a novel extrapulmonary mechanism of gas exchange via peritoneal oxygenated perfluorocarbon (PFC) in a large animal model. METHODS/STUDY POPULATION: Using two 50 kg swine, hypoxia was modeled with subatmospheric oxygen and hypercarbia induced with acute hypoventilation. Through a midline laparotomy, cannulas were placed into the peritoneal space to allow for PFC infusion and circulation. After abdominal closure, these cannulas were connected to a device capable of draining, oxygenating, and infusing PFC. One animal was subjected to acute hypoxia (12% FiO<sub>2</sub>) and another animal to acute hypoventilation (4 breaths per minute). Primary outcomes were times for SpO<sub>2</sub> to reach 75 mmHg, respectively. Trials were performed without PFC and with PFC dwelling or circulating through the peritoneal space, during which abdominal and bladder pressures were monitored and maintained under 20 mmHg by regulation of the PFC volume contained in the animal. RESULTS/ANTICIPATED RESULTS: In the animal subjected to acute hypoxia (12% FiO<sub>2</sub>), survival time improved from 5:55 to 20:00 (min:sec) after 2.5 liters of oxygenated PFC was instilled in the peritoneal space. Oxygen percent saturation of PFC before and after dwelling in the peritoneal space was measured at 100%

before and 70% after dwelling in the animal during this hypoxic period corresponding with a gas transfer of 300 mL of oxygen over the 20-minute trial (i.e., 15 mL/min). Continual PFC circulation did not further extend the survival time during hypoxic conditions over PFC dwelling in the abdomen. In the animal that was acutely hypoventilated, there were no detectable differences in the rate of CO<sub>2</sub> accumulation as measured by EtCO<sub>2</sub> or direct blood pCO<sub>2</sub> measurements with PFC dwelling or circulating through the peritoneal space. DISCUSSION/SIGNIFICANCE: Oxygenated PFC dwelling in the peritoneal space increased the duration of systemic arterial blood saturation remaining greater than 50% during normobaric hypoxic (12% FiO<sub>2</sub>) conditions but did not appreciably clear blood carbon dioxide during hypoventilation. Future experiments will focus on maximizing the rate of systemic oxygen uptake.

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### Paired associative stimulation: a tool for assessing sensorimotor neural signaling and lower limb function post-stroke<sup>+</sup>

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OBJECTIVES/GOALS: A stroke can impair neural communication between sensory and motor pathways thus compromising walking function. Paired associative stimulation (PAS) is a useful assay of sensorimotor integration (SMI) with limited use post-stroke. The objective of this study will be to determine lower extremity PAS effectiveness and reliability post-stroke. METHODS/STUDY POPULATION: This study will use a pre-post, cross-sectional design. Ten healthy controls and 10 individuals with chronic stroke (>6 months) will be recruited. PAS protocols will be individualized to account for between-subject variability in sensorimotor signaling by first measuring cortical sensory signaling using electroencephalography. Post-stroke participants will then receive PAS targeting the paretic tibialis anterior muscle; healthy controls will receive PAS targeting the non-dominant TA. Changes in cortically derived muscle responses will be characterized by absolute motor-evoked potential amplitude (MEPamp) change, elicited by transcranial magnetic stimulation, over two sessions separated by >24 hours. Clinical measures of sensorimotor function and walking ability will also be performed. RESULTS/ANTICIPATED RESULTS: By individualizing PAS protocols, we expect to see significant increases in MEPamp pre to post PAS, determined using paired t-tests. We also anticipate reliable PAS-induced increases in MEPamp, which will be assessed using two reliability statistics: intraclass correlation coefficient and coefficients of variation of method error. Lastly, the increases in MEPamp will be correlated with measures of sensorimotor function and walking ability, anticipating that greater increases in MEPamp will be related to better walking ability and sensorimotor functioning. Correlations will be assessed via a Pearson's correlation. A preset alpha = 0.05 will be used to determine significant findings. DISCUSSION/SIGNIFICANCE: The importance of this study is that establishing individualized PAS protocols could potentially provide a reliable and clinically relevant measure of SMI. Understanding post-stroke lower extremity SMI is necessary for furthering targeted and personalized interventions to combat walking deficits.

<sup>+</sup>Kirstin-Friederike Heise has been added as an author. An addendum detailing this change has also been published (doi:10.1017/cts.2023.563).