SUPPLEMENTARY METHODS

INSTITUTIONAL REVIEW BOARD APPROVAL. Patients were recruited as part of the Injured Spinal Cord Pressure Evaluation (ISCoPE) and Duroplasty for Injured cervical Spinal Cord with Uncontrolled Swelling (DISCUS) studies at St George's Hospital. Approval for ISCoPE was granted from the St George's, University of London Joint Research and Enterprise Service and the National Research Ethics Service London - St Giles Committee (10/H0807/23). Approval for DISCUS was granted from the St George's, University of London Joint Research and Enterprise Service and the Health Research Authority London - Camberwell St Giles Research Ethics Committee (21/LO/0216). Both studies are conducted in accordance with the ethical standards, as agreed in the 1964 Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from all participants. The non-traumatic spinal cord injury spinal patients and the two patients that underwent craniotomies were consented for intraoperative photography, which is part of the standard surgical consent process.

CRANIOTOMY PATIENTS. We recruited two patients who underwent craniotomies as controls, to investigate whether LSCI produces expected changes in cerebral blood flow in response to different maneuvers. LSCI was used in a 47-year-old female patient undergoing left pterional craniotomy, for clipping of a ruptured anterior communicating artery aneurysm, to investigate the effect of sevoflurane MAC on cerebral blood flow. In addition, LSCI was used in a 59-year-old male patient undergoing left frontal craniotomy, for arteriovenous malformation extirpation, to investigate the effect of change in P_aCO_2 on cerebral blood flow.

INCLUSION AND EXCLUSION CRITERIA. All traumatic spinal cord injury patients recruited into DISCUS and ISCoPE between January 2023 and January 2025 were included.

Cervical (C2–T1) injuries were recruited to DISCUS and thoracic (T2–L1) injuries were recruited to ISCoPE. Inclusion criteria: 1) severe traumatic spinal cord injury (American spinal injury association Impairment Scale [AIS] grade A-C), 2) age above 16 years for DISCUS and 18–70 for ISCoPE, 3) surgery necessitating laminectomy and 4) timing of surgery within 72 hours of injury. Exclusion criteria: 1) traumatic dural tear, 2) significant comorbidities, 3) multilevel injuries and 4) other central nervous system disease. Non-traumatic spinal cord injury patients undergoing elective posterior spinal laminectomy were recruited between January 2023 and June 2025. Patients were aged 18 to 80 and had American Society of Anesthesiologists (ASA) score 1 or 2. Patients with pre-existing cardiovascular disease, respiratory disease, ASA ≥3, and patients who had stroke or other intracranial pathology were excluded.

INTRAOPERATIVE ANAESTHESIA. All patients underwent invasive radial artery line insertion, electrocardiogram (ECG), arterial hemoglobin O_2 saturation, end-tidal CO_2 ($EtCO_2$) and bispectral index (BIS) monitoring intraoperatively. Patients received either total intravenous anesthesia with target-controlled infusion of propofol and remifentanil, or inhalational anesthesia with sevoflurane at minimum alveolar concentration (MAC) targets of 0.7, 1.0 or 1.5, at the discretion of the anesthesiologist. Muscle relaxation was achieved with rocuronium, and patients underwent endotracheal intubation. An intravenous infusion of metaraminol was used to maintain MAP at ±10% of the preoperative baseline, and patients underwent endotracheal intubation. BIS was maintained between 40–60 from induction to ensure no anesthetic awareness. Core body temperature was kept 36–37 ºC. Patients were ventilated with an O_2-air mixture and ventilation parameters were adjusted by a theater anesthetic machine (Perseus A500; Dräger, Lubeck, Germany) to maintain a partial pressure of arterial O_2 (P_aO_2) >13 kPa and P_aCO_2 5.0–5.5 kPa, unless P_aCO_2 was adjusted as stated in

the protocols. Isotonic intravenous infusions were used for fluid maintenance, and no osmotic diuretics were administered. Intraoperative physiological data were sampled at 500 Hz using a laptop running Intensive Care Monitor plus (ICM+) software (Cambridge Enterprise ICM+, Cambridge, United Kingdom) which collected arterial line blood pressure, ECG lead II, $EtCO_2$, end-tidal sevoflurane and BIS waveforms from the anesthetic Philips monitor (Intellivue MX700; Philips, Guildford, United Kingdom).

INTROPERATIVE PROTOCOLS. After surgical resection and decompression, we exploited various intraoperative protocols routinely used in our department to address our questions:

1. Spinal cord autoregulation – before wound closure, surgeons ask for the MAP to be increased to confirm hemostasis. MAP is typically elevated from 70–80 mmHg to 100–110 mmHg. Wherever possible, LSCI was done whilst maintaining constant $EtCO_2$ and anesthetic depth (confirmed with BIS) during such MAP elevation.
2. Spinal cord CO_2 reactivity – whilst maintaining constant MAP and anesthetic depth, ventilation parameters were adjusted to achieve P_aCO_2 levels low (3.9–4.2 kPa), normal (5.0–5.5 kPa), and high (6.5–7.0 kPa) to determine optimum ventilatory parameters for intensive care. Whenever possible, LSCI recording was obtained for 20 s during P_aCO_2 steady state.
3. Anesthetic dose – Sevoflurane MAC was adjusted to 0.7, 1.0 or 1.5 at the discretion of the anesthesiologist whilst maintaining constant $EtCO_2$ and MAP. Whenever possible, LSCI recording was obtained for 20 s during sevoflurane steady state.
4. Irrigation fluid temperature – different surgeons prefer different irrigation fluid temperatures. The surgical cavity was flushed with 50 mL of cool, room temperature and warm saline irrigation whilst maintaining a constant $EtCO_2$, MAP and anesthetic depth. After aspiration of irrigation fluid, cerebrospinal fluid (CSF) and blood from

the surgical cavity, LSCI recording was obtained for 20 s. Cool irrigation saline was kept in the operating theater refrigerator at a temperature of 4 ºC, room temperature irrigation saline was stocked in the theater storage shelves at 20 ºC, and warm temperature irrigation saline was kept in a fluid warming cupboard set to 45 ºC. Irrigation saline was used within 2 min of extraction from storage.

5. Neurovascular coupling – all intradural spinal cord lesion resections are operated under intraoperative neuromonitoring with peripheral and direct spinal cord stimulation. After spinal cord decompression, integrity of the dorsal column medial lemniscus tracts was checked with somatosensory evoked potential amplitude measurement after direct spinal cord tissue stimulation at 0.5mA and 1Hz frequency (ISIS Xpert plus; Inomed, Emmendingen, Germany). Wherever possible, LSCI was done whilst maintaining constant $EtCO_2$, anesthetic depth (confirmed with BIS or end-tidal sevoflurane) and MAP, during stimulation.

Patient inclusion and the choice of which studies to perform on each patient were largely based on practical constraints, e.g. availability of operating theater time, and anesthesiologist's preference for type of anesthesia.

MONITORING SCBF. A portable LSCI machine with a near-infrared laser diode at 785 nm (MoorFLPI; Moor Instruments Ltd., Axminster, UK) was mounted onto a trolley by a rigid, extendable arm. During LSCI recording, the tissue surface was kept clear of blood and cerebrospinal fluid (CSF), and background operating theater lighting was switched off. The imager was positioned perpendicularly above the surgical cavity at ~25 cm using fixed-length aiming lasers. Cord perfusion was recorded on a laptop running purpose-designed data acquisition software (MoorFLPI measurement software, Version 3.0; Moor Instruments). Frames were acquired at 748 × 576 pixel resolution, exposed for 20 ms using sliding window

spatial processing at a sampling rate of 25 Hz. These changes allowed for rapid changes of spinal cord perfusion to be determined within a cardiac cycle. The imaging field size was adjusted to include the entire exposed spinal cord. SCBF was displayed as flux in arbitrary units (a.u.). A calibration phantom was used to fix a point on a scale. All measurements were in a.u.'s that cannot be converted to absolute SCBF in mL/100 g/min, nor are they linearly related to absolute SCBF. The software collated and played all frames consecutively thus creating video profiles of tissue SCBF over time. The setup is described in detail in our earlier publications[1, 2] and is summarized in Supplementary Fig. 1 that also shows data recorded intraoperatively from brain.

DATA CLEANING AND ANALYSIS. All data collected from the anesthetic monitor were cleaned of artefact e.g. arterial line flushing and electrical artefact from diathermy. LSCI raw data waveforms of pre-selected regions of interest (ROIs) were exported from the in-house MoorFLPI software to ICM+, via text file format. Where raw data waveforms had artefactual data removed e.g. excessive movement and temporary shadows from surgical instruments. Where possible, ROIs were selected to measure blood flow in tissue (TBF), and within vessels (VBF). Vessel diameter (VD) was also measured using the pixel measurement tool ImageJ (v.1.54p, https://imagej.net/ij/). At least two regions of interest were chosen in each image, this included an area of tissue, and an arterial blood vessel. In traumatic spinal cord injury patients the above 2 ROIs were selected in multiple different regions of perfusion. For each vessel, 10 repeat measurements of vessel diameter were taken during cardiac systole to measure mean values. All ROIs were translated to subsequent LSCI recordings where the investigated parameter was adjusted to enable direct comparisons i.e. anatomical location of each ROI was replicated between each LSCI recording where the investigative parameter was

changed. This sampling method yielded 25 mean flux values per second per ROI thus providing high spatial and temporal resolution.

STATISTICS. A sample size calculation was not possible, because this is an exploratory study. Unless otherwise stated, data are expressed as mean ± 95 % confidence interval. For pairwise comparisons of the effect of different conditions including sevoflurane (MAC 0.7, 1.0, 1.5), P_aCO_2 (low, normal, high), irrigation fluid temperature (low, room, high) on SCBF we used paired Student t-tests with Bonferroni corrections. Statistical significance was taken at $P < 0.05^{*}$, 0.01^{**}, $0.005^{\#}$, $0.001^{\#\#}$.

References

1. Asif H, Boseta E, Zoumprouli A, Papadopoulos MC, Saadoun S: Spinal cord blood flow, metabolism, and neurological outcome in patients with acute, severe traumatic spinal cord injuries. NEUROCRITICAL CARE: In press

2. Gallagher MJ, Hogg FRA, Zoumprouli A, Papadopoulos MC, Saadoun S: Spinal Cord Blood Flow in Patients with Acute Spinal Cord Injuries. J NEUROTRAUMA 2019; 36: 919–29. 10.1089/neu.2018.5961 [doi]