Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Hemorrhage (BREATHE-ICH) Study: Results from an Experimental Interventional Study

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Abstract

Background: Cerebral autoregulation (CA) is impaired in a multitude of neurological conditions. Increasingly, clinical studies are correlating the nature of this impairment with prognostic markers. In acute intracerebral haemorrhage (ICH), impairment of CA has been associated with worsening clinical outcomes including poorer Glasgow Coma Score and larger haematoma volume. Hypocapnia has been shown to improve CA despite concerns over hypoperfusion and consequent ischaemic risks, and it is therefore hypothesized that hypocapnia (via hyperventilation) in acute ICH may improve CA and consequently clinical outcome.

Aims: To assess the feasibility and acceptability of the first CA-targeted intervention in acute ICH utilising a simple bed-side hyperventilatory maneouvre.

Methods: Twelve patients with acute ICH within 48 hours of onset were enrolled. The experimental set-up measured cerebral blood flow velocity (CBFV, transcranial Doppler), blood pressure (BP, Finometer) and end-tidal CO_2 ($EtCO_2$, capnography) at baseline, and in response to hypocapnia (-5mm Hg below baseline) achieved via a 90-second hyperventilatory maneouvre. CA was evaluated with transfer function analysis and autoregulatory index (ARI) calculations.

Results: We observed tolerance to the protocol in a cohort of mild (NIHSS 4) supratentorial ICH patients with small volume haematomas without intraventricular extension. Importantly, a significant difference was noted between ipsilateral ARI at baseline 4.8 (1.7) and ARI during hypocapnic intervention 7.0 (0.8) ($p=0.0004$), reflecting improved CA, though a dose-dependent effect of $EtCO_2$ on ARI was not observed.

Conclusions: In this small study, there was no observed effect on 14-day death and disability in recruited participants. This is the first report of improvement in CA in acute ICH using a non-invasive interventional manoeuvre, through induction of hypocapnia via hyperventilation. (ClinicalTrials.gov Identifier: NCT03324321)

Introduction

Spontaneous acute ICH is associated with both high mortality and morbidity.[1] There is a relative paucity of management options for acute ICH compared to acute ischaemic stroke (AIS) with intensive blood pressure (BP) control to a target systolic BP of 140mmHg within 6 hours of ICH onset recommended by current guidelines.[2, 3] However, a key limitation of large-scale randomized controlled trials has been the inability to provide mechanistic insight into cerebral blood flow velocity (CBFV) during the acute phase of haemorrhagic stroke.[2, 4] This is particularly the case if CBF control mechanisms are altered by chronic hypertension or indeed ICH itself.

Cerebral autoregulation (CA) provides a protective mechanism for possible haematoma expansion or perihaematomal ischemia, secondary to hypertension/hyperperfusion or hypotension/hypoperfusion, respectively. Dynamic CA (dCA) is a measure of the response of CBFV to rapid BP changes, and several key studies have shown impaired dCA post-acute ICH.[5-9] A recent meta-analysis demonstrated that dCA impairment lasts up to 12 days following supratentorial ICH, and that CA is bilaterally disturbed.[10] Importantly a recent study showed larger haematoma volume is likely to independently predict poorer CA status ipsilateral to the haematoma.[9]

Hypocapnia in healthy volunteers leads to improvements in CA.[11, 12] Furthermore, hypocapnia, typically through hyperventilation in intubated patients, has been used in the management of acute brain injury to reduce intracranial pressure (ICP) and to improve outcome in specific circumstances.[13, 14] The reduction in ICP may reduce the risk of secondary brain injury from direct pressure or by brainstem herniation.[14] However, hypocapnia induces lower ICP by decreasing the CBF via cerebral arterial vasoconstriction, with CBF decreases of approximately 3% per mmHg change in $PaCO_2$ (range, 60 to

71 20mmHg $PaCO_2$) reported in patients with traumatic brain injury (TBI).[13, 15] This risks
72 hypoperfusion and subsequent neuronal ischaemia, which could worsen outcome.[16]

73 Therefore, whilst improving dCA by means of hyperventilation in acute ICH offers a novel
74 non-pharmacological intervention, further evaluation is required to ensure overall beneficial
75 and not detrimental effects on cerebral haemodynamics in acute ICH.

76 Aims

77 To assess the feasibility and acceptability of the first CA-targeted intervention in acute ICH
78 utilising a simple bed-side hyperventilatory manoeuvre.

79 Accordingly, the BREATHE-ICH study will assess if: i) ICH sufferers will tolerate a
80 hyperventilation manoeuvre; ii) a hyperventilation manoeuvre produces hypocapnia in an
81 ICH population; and iii) it is feasible to improve dCA (with 'responders' demonstrating lower
82 CBFV though improved ARI), whilst maintaining safe levels of CBFV.

Materials and Methods

Ethics statement

The East Midlands – Nottingham 1 Research Ethics Committee (REC) provided ethical approval for the study (17/EM/0283). Informed consent was obtained from participants in line with recommendations set out in the E6 (R1) of the International Council for Harmonization of Technical Requirements or Pharmaceuticals for Human Use (ICH) Good Clinical Practice Guidelines. All patients provided written informed consent and all study procedures were completed in accordance with the most recent revision of the Declaration of Helsinki.[17]

Sample Selection

The protocol for the BREATHE-ICH study was prospectively published.[18] Acute ICH patients, able to comply with a respiratory manoeuvre (hyperventilation) were recruited within 48 hours of onset. Each stroke patient was required to participate for up to 14 days post stroke symptom onset; a maximum of two cerebral haemodynamic assessments being made. The first assessment was recorded whilst the participant was an in-patient under the care of the University Hospitals of Leicester NHS Trust Stroke Service (acute: within 48 hours of symptom onset); the second (sub-acute) assessment being undertaken 10-14 days post stroke onset.

Patients with acute ICH meeting the inclusion and exclusion criteria were considered for enrolment between October 2017 and July 2018. Inclusion criteria were a clinical diagnosis of haemorrhagic stroke within 48 hours of onset (for patients waking with a stroke, time of onset was taken to be the time when the patient was last asymptomatic), able and willing to give informed consent and competent to comply with a respiratory manoeuvre, as judged by the investigator. Exclusion criteria included those with significant previous airways disease (formal diagnosis of moderate or severe airways disease and having treatment for this

respiratory condition – via inhalers or specialist input) and co-morbidity with anticipated life expectancy less than 3 months.

Intervention

dCA measurements were performed during a hyperventilation protocol. This involved sustained periods of 90-seconds of hyperventilation at -5mmHg below baseline $EtCO_2$, regulated using a metronome. The patients received a demonstration from the conducting physician clarifying the expected relationship of their breathing with the metronome beat. Two-minute washout periods of normal respiration were allowed between successive measurements. Each incremental reduction in $EtCO_2$ was repeated on two occasions during the same session. Patients underwent repeated assessments of hypocapnia with intervening periods of washout, i.e. two repetitions of the hypocapnic intervention with interval 5-minute baselines. This provided the opportunity to refine the compliance with the metronome and ensure the manoeuvre was successfully achieved on at least one occasion per assessment period.

Participants were monitored throughout the process and any unexpected physiological changes were recorded on an adverse event form. In preparation for the study, lower limits of acceptable $EtCO_2$ (29 mmHg) and CBFV (33 cm/s) calculated as within 1 SD from the mean hypocapnia level for cohort of healthy volunteer values[12] and based on previous acceptable limits in TBI studies.[19] This helped protect individuals from risk of ischaemia associated with hypocapnia-induced vasoconstriction.

Interpretation

The study was designed to test the feasibility of a CA-targeted intervention using hyperventilation. Therefore the primary outcome of interest was the proportion of recruited individuals able to comply with the full measurement protocol. Data were assessed for quality

during the analysis protocol, and rejected data files recorded with associated reasoning. Percentage change in baseline and in response to a hypocapnic manoeuvre in the acute and sub-acute phase were recorded alongside ARI (see below). 'Responders' were defined as those individuals for whom lower CBFV and improvement in ARI were demonstrated during hypocapnic conditions. 'Non-responders' were defined as those from whom this expected physiological response was not demonstrated. In addition, classical end-points associated with this before and after interventional study, included death and disability at 14 days, were also recorded.

Assessment of Hemodynamic Function

All assessments were undertaken in the *Cerebral Haemodynamics in Ageing and Stroke Medicine* cardiovascular research laboratory at the Leicester Royal Infirmary (LRI), which is at a controlled temperature (20-24°C) and is free from distraction. Where the patient was medically unstable and unable to be transferred to the laboratory, assessments were undertaken in the hyperacute stroke bay. The subject lay supine on an examination couch. Baseline casual BP was calculated as a mean of three supine brachial BP readings using a validated UA767 BP monitor. Beat-to-beat non-invasive BP were recorded continuously using the Finometer (FMS, Netherlands) cuff device attached to the middle finger of the non-hemiparetic hand. R-R interval was recorded using a 3-lead ECG. Respiratory rate was recorded, and end-tidal partial pressure of carbon dioxide ($EtCO_2$) monitored using small nasal cannulae placed at the base of the nose (Salter Labs, ref 4000) attached to a capnograph (Capnocheck Plus). Simultaneous bilateral insonation of the middle cerebral arteries (MCAs) was performed with the subject lying supine on a couch using transcranial Doppler ultrasound (TCD, DWL Dopplerbox 10.5.1 software version) to measure CBFV. Using 2MHz probes, supported by a custom-made frame, the vessel was located via the temporal bone window, and identified as the MCA by the waveform, its depth, velocity, and the

direction of flow. All parameters were simultaneously recorded onto a computer data acquisition system (PHYSIDAS), for subsequent off-line analysis.

Assessment of Haemorrhage Volume, Intraventricular and Subarachnoid Extension

A CT scan within 48 hours was analysed for haematoma size, intraventricular and subarachnoid extension. The assessment of these imaging parameters was performed, blinded to any CA data from initial CT or magnetic resonance imaging, according to the *ABC* criteria (assess the length, width and depth of haemorrhage in centimetres) associated with the shape of the haemorrhagic lesion.[20] The ICH volume was calculated from the first CT scan using the *a x b x c x 0.5 (ABC/2)* method. The CT imaging underwent adjudication by an independent neuroradiologist (DS).

Assessment of Clinical Parameters

At admission, demographic, baseline clinical and laboratory parameters including age, sex and history of hypertension, previous antihypertensive therapy, National Institutes of Health Scale (NIHSS) score, and BP were recorded.

Data Analysis

Transfer function analysis (TFA) is a widely used and validated technique of examining the relationship between CBFV and BP, and allows quantification of the close transfer of spontaneous BP fluctuations to CBFV, providing an objective measure of dCA. Furthermore, TFA and coherence (linearity between input and output signals) was calculated using Welch's method; coherence determining acceptability of good quality data taking into consideration appropriate number of degrees of freedom.[21] The first harmonic method was used to estimate critical closing pressure (CrCP) and resistance-area product (RAP). Lastly, to derive ARI, a time-domain approach was used as described by Tiecks *et al.*[22] from a

standardized set of curves based on CBFV response to sudden step changes in BP following thigh cuff deflation.

Statistical Analysis

All normally distributed continuous variables are described as mean (SD) and non-Gaussian with skewness as median (IQR), where appropriate. Comparison of baseline data in acute ICH patients was made using the Student t-test for normally distributed data, with Bonferroni's correction for multiple comparisons as appropriate, or by appropriate non-parametric tests. Values of $p<0.05$ were considered statistically significant. All statistics were performed using statistical software GraphPad Prism 7 for Windows.

189 # Results

190 Altogether 18 acute ICH patients met the inclusion criteria. Five patients were excluded due
191 to lack of acceptable TCD windows and one further patient was excluded due to CT
192 confirmation at a later date of haemorrhagic transformation of cerebral infarction, as opposed
193 to primary acute ICH (Supplemental Material Figure I). Therefore, a total of 12 patients of
194 mean age 68 (range 30 to 91) years, and mean haemorrhage volume of 5.78 mL (range 0.21
195 to 14.76) were included in the analysis. No patients had intraventricular extension, though
196 2/12 (17%) had subarachnoid extension. Detailed demographic, clinical and radiological
197 characteristics of the study population are reported in Table 1. Follow-up assessments were
198 performed on 9 patients; 3 patients were resident in local community hospitals and not able to
199 return for repeat assessments due to ongoing stroke specific rehabilitation needs.

200 The intervention was tolerated without any adverse clinical consequences including
201 headache, syncope, worsening of NIHSS/new stroke symptoms or any hypocapnia-related
202 paresthesia.

203 ***Results BREATHE-ICH Intervention and Haemodynamics at <48 hours***

204 The responses pre- and post-hypocapnic interventions are reported in Table 2 and in Figs. 1
205 and 2. There was no significant difference between baseline $EtCO_2$ (35.4 mmHg) and $EtCO_2$
206 for hypocapnic intervention (32.4 mmHg) (p=0.08, Table 1). However, a significant
207 improvement was noted, despite the lack of significant reduction in $EtCO_2$, between
208 ipsilateral ARI at baseline 4.8 (1.7) and ARI during hypocapnic intervention 7.0 (0.8)
209 (p=0.0004, Table 1).

210 Individualised plots demonstrating variability in response for the 12 patients undergoing
211 initial assessment (<48 hours) post-acute ICH for ipsilateral and contralateral CBFV and ARI

212 are shown in Figure 1. In addition, individualised delta (Δ) $EtCO_2$ change, and corresponding
213 delta (Δ) CBFV and ARI changes are demonstrated in Figure 2. These provide an overview
214 of the changes observed in each individual in response to the intervention of $EtCO_2$ lowering.
215 Figure 3 demonstrates the significant variability in individual assessments with 11
216 'responders' demonstrating the lower CBFV and improved ARI in response to lower $EtCO_2$.
217 Both *Patient 6* and *Patient 11* were 'non-responders' for CBFV and *Patient 7* for ARI, with
218 *Patient 2* exhibiting no ARI change in response to a -3mmHg $EtCO_2$ decrease.

219 ***Results BREATHE-ICH Intervention and Haemodynamics at 10-14 days***

220 Follow-up haemodynamic data pre- and post-hypocapnic intervention at 10-14 days post-
221 acute ICH are reported in Table 3. Furthermore, individualised plots demonstrating
222 variability in response for the 9 patients followed-up (10-14 days) post-acute ICH for
223 ipsilateral and contralateral CBFV and ARI are shown in Figure 3. In addition, baseline ARI
224 was examined relative to haematoma volume, demonstrating visually the relationship
225 between haematoma size and ARI values (Supplemental Material Figure II).

226

227 **Discussion**

228 ***Key Findings***

229 The BREATHE-ICH study demonstrated: i) ICH sufferers tolerate a hyperventilatory
230 manoeuvre; ii) a hyperventilation manoeuvre produces hypocapnia in an ICH population; and
231 iii) it is feasible to improve dCA (with 'responders' demonstrating lower CBFV though
232 improved ARI), whilst maintaining safe levels of CBFV. Overall, the study demonstrated
233 feasibility of manipulating $EtCO_2$ to improve ipsilateral dCA in a cohort of mild (NIHSS 4)
234 supratentorial ICH patients with small volume haematomas without intraventricular
235 extension.

236 ***Literature Context***

237 A meta-analysis of eight previous cerebral haemodynamic studies (293 ICH patients) in acute
238 ICH demonstrated impaired CA using different CA indices, including reduced TFA phase,
239 higher mean flow correlation and correlation of these parameters with poor prognostic
240 markers of ICH. However, no prior studies had used the CA index ARI or indeed aligned
241 CO_2 change with CA status.[10] The demonstration of ipsilateral improvement in ARI and the
242 lack of significant adverse physiological change during individualised assessment of
243 responses to this interventional protocol is a significant step beyond current understanding.
244 The careful process of translation from healthy volunteers to ICH patients has involved
245 several steps in both logistical and scientific understanding as to how $PaCO_2$ change
246 influences CBFV and autoregulatory function.[10, 12, 18, 23-25] The inclusion of individuals with
247 variable locations of ICH is a strength (cortical and lobar involvement), however, this does
248 assume that ICH generally occurs through similar pathophysiological mechanisms
249 irrespective of location. Differences in CA across differing ICH subtypes has yet to be
250 assessed within the literature.

251 ***Intervention Feasibility***

The data supports an individualised response by patients suffering an ICH, with ARI at or below the impaired cut-off of <4, improving towards ARI values in excess of 4.[26] Furthermore, this study affirms prior observational physiological measurement stroke studies that demonstrate the presence of hypocapnia post-stroke. The lack of a clear stepwise drop between averaged $EtCO_2$ values is likely associated with statistical power, though assessment of individualised responses to the intervention demonstrates the feasibility of lowering $EtCO_2$ in acute ICH (11/12 patients). Importantly, despite a small patient cohort, there was a significant difference in ARI despite a lack of significant reduction in $EtCO_2$ at a whole study population level. Despite hyperventilation-induced hypocapnia originally being used in intubated patients with high ICP as an intervention to lower ICP, two further applications could also be considered for hypocapnia in an ICH patient population. Firstly, chronic hypertension shifts the autoregulatory curve to the right with less of a plateau and subsequent ability to respond to changes in cerebral perfusion pressure, hypocapnia could shift the curve to minimize the effect of chronic hypertension. Secondly, early in ICH, vasoconstriction induced by hyperventilation associated hypocapnia may provide a means of minimising haematoma expansion via a vasoconstrictive/vasoparalysis based intervention targeting the bleeding vessel.

CA and Hypocapnia at <48 hours in the BREATHE-ICH Study

The demographic of patients recruited to the study were comparable to prior dCA in acute ICH studies with similar age, systolic BP, diastolic BP, and haematoma locations.[10] Arguably, the severity was greater (NIHSS >10) in prior studies, however, these studies were not designed to deliver an intervention to this patient population. Furthermore, haematoma volumes were smaller than the other dCA studies in ICH for the same reason, though almost a fifth of the cohort had evidence of subarachnoid extension, a poor prognostic feature in ICH.[27]

The demonstration of a 'response' on individualised assessment (Figure 2) of all participants except two (83.3%) is encouraging. This supports the translation of the physiologically expected response from healthy volunteers to those with acute ICH. Furthermore, the lack of any significant adverse physiological change, aside from a dramatic drop in CBFV for *Patient 2* of 36 $cm.s^{-1}$ with a lack of ARI improvement. This could be explained by the presence of chronic hypertension (with three prior anti-hypertensive drugs being taken) and ongoing acute BP lowering therapy being delivered (i.e. Labetolol or Glyceryl Trinitrate therapy) as anti-hypertensive therapy has been demonstrated to affect both BP variability and autoregulatory response in both a positive and negative manner.[28-30]

CA and Hypocapnia at 10-14 days in the BREATHE-ICH Study

During the follow-up period, the significant difference in $EtCO_2$ between the baseline measurement and the intervention suggests ch protocol was better implemented in less acutely unwell patients after 10-14 days post stroke. Importantly, though no significant benefit (but also no harm) was seen in this assessment period (as compared to the acute period), this is important to explore in a larger group and to consider alternative options to reduce $PaCO_2$ in acutely unwell patients, particularly those with larger haematoma volumes or a more severe NIHSS.[6, 9] Studies in animal models of subarachnoid haemorrhage (SAH) have concluded that post haemorrhagic microspasm leads to complete loss of CO_2 reactivity for up to 24 hours and therefore FV cannot be increased during hypercapnia.[33]

Limitations

Firstly, these results alongside those of prior studies in ICH have indicated that the pathophysiology of dCA in ICH patients is complex. Despite prior work demonstrating ipsilateral autoregulation is closely correlated with haematoma volume, contralateral autoregulation of ICH can also provide information about the presence of small vessel disease and baseline intracranial leukoaraiosis and therefore may not be a reliable marker of

302 preceding autoregulation status.[9] Furthermore, if the sample size had been larger and nearer
303 the second power threshold (n=40), a more accurate assessment of other parameters aside
304 from ARI may have been obtained.

305 Secondly, there was evidence of significant selection bias, towards mild to moderate ICH as
306 dCA monitoring was not considered possible in severe ICH, particularly with the
307 involvement of a ventilatory maneouvre. The mean haematoma volume of 5.78ml and NIHSS
308 of 4 are not usual for ICH and given the broad inclusion criteria demonstrate selection bias
309 during recruitment. Importantly, the included patient population are less likely to experience
310 haematoma expansion or overall a poorer prognosis post ICH, application of the intervention
311 to a consecutive cohort of all admitted ICH patients would be necessary to understand the
312 true feasibility of the intervention. Overall, this limitation prevents generalizability of
313 findings to those with larger haematomas, higher NIHSS scores and lower level of
314 consciousness.

315 Thirdly, we did not directly assess the effects of BP-lowering therapy on dCA, so there will
316 remain limited data on the impact of intensive BP lowering on cerebral haemodynamics.
317 Therefore, a mechanistic understanding from a cerebral haemodynamic perspective of
318 different targets for intensive BP lowering is lacking. Fourthly, use of nasal cannulae for
319 $PaCO_2$ measurement has benefits associated with rapid response time to changes, though
320 concerns exist over accuracy compared to face mask measurements.[24] There are no studies to
321 date showing MCA diameter change with hypocapnia despite evidence being shown in
322 extreme hypercapnia.[44]

323 Fourthly, the study could be improved using isocapnic clamping during baseline periods to
324 ensure the exact deviation of $EtCO_2$ during hypocapnia is deduced. However, as
325 demonstrated in prior studies, there is an acceptable level of accuracy using non-invasive

326 $EtCO_2$ assessment with capnography at the bedside [24]. Despite the benefits of repeating the
327 hypocapnic intervention to ensure compliance and therefore to ensure that the strength of the
328 response was optimized, there remains the possibility of inadequate washout and hence
329 ongoing vasoconstrictive processes on a microvascular level with biochemical processes
330 governing reversibility post hypocapnia.

331 Dynamic CA can be improved in acute ICH using an interventional manoeuvre, namely
332 generating hypocapnia via hyperventilation. This was demonstrated to be safe and feasible
333 with no adverse clinical outcomes. Further studies are required to examine the possibility of
334 improving clinical outcomes at 30 days, as well as the assessment of alternative methods in a
335 more severe ICH population that would be less able to comply with a hyperventilatory
336 manoeuvre.

337 Further exploration beyond haematoma volume in larger clinical cohorts would be useful to
338 provide further perspectives on associations between CA and poor prognostic markers
339 demonstrated in prior work.[10] The results provide a platform foundation for larger trials,
340 which can focus on outcomes for 'responders' and 'non-responders' to the intervention. If
341 this distinction is made during initial clinical assessment post-acute ICH, it may provide a
342 basis for offering personalized interventions to specific ICH sufferers in an effort to expand
343 current treatment options.

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Author Contributions

JSM, RBP and TGR designed the study. JSM performed the experiments. JSM and RBP analyzed the experiments. DS analyzed the clinical imaging files. JSM, RBP, DS and TGR wrote the manuscript. The data that support the findings of this study are available from the first author (jm591@le.ac.uk) upon reasonable request.

References

1. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol.* 2010;9:167-176.

Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. *N Engl J Med.* 2013;20;368:2355-2365.

3. Steiner T, Al-Shahi Salman R, Beer R, Christensen H, Cordonnier C, Csiba L, et al. European Stroke Organisation (ESO) guidelines for the management of spontaneous intracerebral hemorrhage. *Int J Stroke.* 2014;9:840-855.

4. Qureshi AI, Palesch YY, Barsan WG, Hanley DF, Hsu CY, Martin RL, et al. Intensive Blood-Pressure Lowering in Patients with Acute Cerebral Hemorrhage. *N Engl J Med.* 2016;375:1033-1043.

5. Reinhard M, Neunhoeffer F, Gerds TA, Niesen WD, Buttler KJ, Timmer J, et al. Secondary decline of cerebral autoregulation is associated with worse outcome after intracerebral hemorrhage. *Intensive Care Med.* 2010;36:264-271.

6. Nakagawa K, Serrador JM, LaRose SL, Sorond FA. Dynamic cerebral autoregulation after intracerebral hemorrhage: A case-control study. *BMC Neurol.* 2011;11:108-2377-11-108.

7. Oeinck M, Neunhoeffer F, Buttler KJ, Meckel S, Schmidt B, Czosnyka M, et al. Dynamic cerebral autoregulation in acute intracerebral hemorrhage. *Stroke.* 2013;44:2722-2728.

382 8. Ma H, Guo ZN, Liu J, Xing Y, Zhao R, Yang Y. Temporal Course of Dynamic Cerebral
383 Autoregulation in Patients With Intracerebral Hemorrhage. *Stroke.* 2016;47:674-681.

384 9. Ma H, Guo ZN, Sun X, Liu J, Lv S, Zhao L, et al. Hematoma volume is a predictive factor
385 of disturbed autoregulation after spontaneous intracerebral hemorrhage. *J Neurol Sci.*
386 2017;382:96-100. doi: S0022-510X(17)34342-3.

387 10. Minhas JS, Panerai RB, Ghaly G, Divall P, Robinson TG. Cerebral autoregulation in
388 hemorrhagic stroke: A systematic review and meta-analysis of transcranial Doppler
389 ultrasonography studies. *J Clin Ultrasound.* 2019;47:14-21.

390 11. Dineen NE, Brodie FG, Robinson TG, Panerai RB. Continuous estimates of dynamic
391 cerebral autoregulation during transient hypocapnia and hypercapnia. *J Appl Physiol.*
392 2010;108:604-613.

393 12. Minhas JS, Panerai RB, Robinson TG. Modelling the cerebral haemodynamic response in
394 the physiological range of $PaCO_2$. *Physiol Meas*. 2018;39:065001-6579/aac76b.

395 13. Neumann JO, Chambers IR, Citerio G, Enblad P, Gregson BA, Howells T, et al. The use
396 of hyperventilation therapy after traumatic brain injury in Europe: an analysis of the BrainIT
397 database. *Intensive Care Med*. 2008;34:1676-1682.

398 14. Laffey JG, Kavanagh BP. Hypocapnia. *N Engl J Med*. 2002;347:43-53.

399 15. Brian JE,Jr. Carbon dioxide and the cerebral circulation. *Anesthesiology.* 1998;88:1365-
400 1386.

401 16. Minhas JS. Highlighting the Potential Value of Capnography in Acute Stroke. *J Emerg*
402 *Med.* 2018;55:130-131.

403 17. World Medical Association. World Medical Association Declaration of Helsinki: ethical
404 principles for medical research involving human subjects. *JAMA*. 2013;310:2191-2194.

405 18. Minhas JS, Panerai RB, Robinson TG. Feasibility of Improving Cerebral Autoregulation
406 in Acute Intracerebral Haemorrhage (BREATHE-ICH) study: a protocol for an experimental
407 interventional study. *BMJ Open.* 2018 Mar 27;8(3):e020758-2017-020758.

408 19. Menon DK, Coles JP, Gupta AK, Fryer TD, Smielewski P, Chatfield DA, et al. Diffusion
409 limited oxygen delivery following head injury. *Crit Care Med* 2004;32:1384-1390.

410 20. Huttner HB, Steiner T, Hartmann M, Kohrmann M, Juettler E, Mueller S, et al.
411 Comparison of ABC/2 estimation technique to computer-assisted planimetric analysis in
412 warfarin-related intracerebral parenchymal hemorrhage. *Stroke*. 2006;37:404-408.

413 21. Claassen JA, Meel-van den Abeelen AS, Simpson DM, Panerai RB, international
414 Cerebral Autoregulation Research Network (CARNet). Transfer function analysis of dynamic
415 cerebral autoregulation: A white paper from the International Cerebral Autoregulation
416 Research Network. *J Cereb Blood Flow Metab*. 2016;36:665-680.

417 22. Tiecks FP, Lam AM, Aaslid R, Newell DW. Comparison of static and dynamic cerebral
418 autoregulation measurements. *Stroke.* 1995;26:1014-1019.

419 23. Minhas JS, Syed NF, Haunton VJ, Panerai RB, Robinson TG, Mistri AK. Is dynamic
420 cerebral autoregulation measurement using transcranial Doppler ultrasound reproducible in
421 the presence of high concentration oxygen and carbon dioxide? *Physiol Meas.* 2016;37:673-
422 682.

423 24. Minhas JS, Robinson T, Panerai R. $PaCO_2$ measurement in cerebral haemodynamics: face
424 mask or nasal cannulae? *Physiol Meas.* 2017;38:N101-N106.

425 25. Minhas JS, Panerai RB, Robinson TG. Sex differences in cerebral haemodynamics across
426 the physiological range of $PaCO_2$. *Physiol Meas.* 2018;39:105009.

427 26. Caldas JR, Panerai RB, Haunton VJ, Almeida JP, Ferreira GS, Camara L, et al. Cerebral
428 blood flow autoregulation in ischemic heart failure. *Am J Physiol Regul Integr Comp Physiol.*
429 2017;312:R108-R113.

430 27. Chen G, Arima H, Wu G, Heeley E, Delcourt C, Zhang P, et al. Subarachnoid extension
431 of intracerebral hemorrhage and 90-day outcomes in INTERACT2. *Stroke*. 2014
432 Jan;45(1):258-260.

433 28. Hoffman WE, Miletich DJ, Albrecht RF. The influence of antihypertensive therapy on
434 cerebral autoregulation in aged hypertensive rats. *Stroke.* 1982;13:701-704.

435 29. Kuriyama Y, Nakamura M, Kyougoku I, Sawada T. Effects of carvedilol on cerebral
436 blood flow and its autoregulation in previous stroke patients with hypertension. *Eur J Clin*
437 *Pharmacol.* 1990;38:S120-1.

438 30. Fu CH, Yang CC, Kuo TB. Effect of angiotensin II receptor antagonism on cerebral
439 vasomotor reserve in humans. *J Cardiovasc Pharmacol.* 2003;42:714-718.

440 31. Conen D, Bertel O, Dubach UC. Cerebral blood flow and calcium antagonists in
441 hypertension. *J Hypertens Suppl*. 1987;5:S75-80.

442 32. Bertel O, Marx BE, Conen D. Effects of antihypertensive treatment on cerebral perfusion.
443 *Am J Med*. 1987;82:29-36.

444 33. Friedrich B, Michalik R, Oniszczuk A, Abubaker K, Kozniewska E, Plesnila N. CO2 has
445 no therapeutic effect on early microvasospasm after experimental subarachnoid hemorrhage.
446 *J Cereb Blood Flow Metab.* 2014;34:e1-6.

447 34. Ma X, Willumsen L, Hauerberg J, Pedersen DB, Juhler M. Effects of graded
448 hyperventilation on cerebral blood flow autoregulation in experimental subarachnoid
449 hemorrhage. *J Cereb Blood Flow Metab*. 2000;20:718-725.

450 35. Xiao M, Li Q, Feng H, Zhang L, Chen Y. Neural Vascular Mechanism for the Cerebral
451 Blood Flow Autoregulation after Hemorrhagic Stroke. *Neural Plast.* 2017;2017:5819514.

452 36. Boarini DJ, Kassell NF, Sprowell JA, Olin JJ, Coester HC. Cerebrovascular effects of
453 hypocapnia during adenosine-induced arterial hypotension. *J Neurosurg*. 1985;63:937-943.

454 37. Norcliffe LJ, Rivera-Ch M, Claydon VE, Moore JP, Leon-Velarde F, Appenzeller O, et
455 al. Cerebrovascular responses to hypoxia and hypocapnia in high-altitude dwellers. *J Physiol.*
456 2005;566:287-294.

457 38. Gupta A, Chazen JL, Hartman M, Delgado D, Anumula N, Shao H, et al. Cerebrovascular
458 reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and
459 meta-analysis. *Stroke*. 2012;43:2884-2891.

460 39. Fierstra J, Spieth S, Tran L, Conklin J, Tymianski M, ter Brugge KG, et al. Severely
461 impaired cerebrovascular reserve in patients with cerebral proliferative angiopathy. J
462 *Neurosurg Pediatr.* 2011;8:310-315.

463 40. Fulesdi B, Limburg M, Bereczki D, Kaplar M, Molnar C, Kappelmayer J, et al.
464 Cerebrovascular reactivity and reserve capacity in type II diabetes mellitus. *J Diabetes*
465 *Complications*. 1999;13:191-199.

466 41. Mirro R, Lowery-Smith L, Armstead WM, Shibata M, Zuckerman SL, Leffler CW.
467 Cerebral vasoconstriction in response to hypocapnia is maintained after ischemia/reperfusion
468 injury in newborn pigs. *Stroke.* 1992;23:1613-1616.

469 42. Golding EM, Steenberg ML, Contant CF,Jr, Krishnappa I, Robertson CS, Bryan RM,Jr.
470 Cerebrovascular reactivity to $CO_{(2)}$ and hypotension after mild cortical impact injury. *Am J*
471 *Physiol*. 1999;277:H1457-66.

472 43. Lingappan K, Kaiser JR, Srinivasan C, Gunn AJ. Relationship between PCO_2 and
473 unfavorable outcome in infants with moderate-to-severe hypoxic ischemic encephalopathy.
474 Pediatr Res 2016 Aug;80(2):204-208.

475 44. Coverdale NS, Gati JS, Opalevych O, Perrotta A, Shoemaker JK. Cerebral blood flow
476 velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J*
477 *Appl Physiol.* 2014;117:1090-1096.

478 45. Brodie FG, Atkins ER, Robinson TG, Panerai RB. Reliability of dynamic cerebral
479 autoregulation measurement using spontaneous fluctuations in blood pressure. *Clin Sci*
480 *(Lond).* 2009;116:513-520.

481

482 # Tables

483 **Table 1:** Demographic and baseline characteristics.

	Subjects (n=12)
Age, y	68 (16)
Sex, male/female	8/4
Time to first assessment, hours	24 (11)
Systolic BP on admission, mmHg	160 (30)
Diastolic BP on admission, mmHg	87 (17)
NIHSS on admission, points	4 (1-13)
NIHSS on assessment, points	4 (3)
GCS , n (range)	15 (14-15)
Hematoma side, left/right	7/5
Hematoma location, n (%)	
Basal ganglia	2 (17)
Thalamus	3 (25)
Lobar	7 (58)
ICH volume, mL	5.78 (4.96)
Intraventricular haemorrhage, n (%)	0 (0)
Subarachnoid haemorrhage, n (%)	2 (17)
Hypertension, n (%)	5 (42)
Diabetes mellitus, n (%)	1 (8)
Hyperlipidemia, n (%)	1 (8)
Smoking, n (%)	1 (8)
Excessive drinking, n (%)	0 (0)

484 Values are mean (SD) unless otherwise stated

485 **Table 2:** Haemodynamic characteristics at baseline assessment and during intervention (<48 hours).

	Baseline	Hypocapnic Intervention	p-value
$EtCO_2$ (mmHg)	35.4 (4.0)	32.4 (4.2)	0.08
CBFV Ipsilateral ($cm.s^{-1}$)	49.2 (18.7)	44.0 (12.6)	0.44
CBFV Contralateral ($cm.s^{-1}$)	50.1 (15.8)	45.7 (14.1)	0.54
ARI Ipsilateral	4.8 (1.7)	7.0 (0.8)	**0.0004**
ARI Contralateral	4.7 (2.1)	5.9 (1.7)	0.21
Mean BP (mmHg)	102.9 (15.5)	95.2 (13.0)	0.20
Heart Rate (bpm)	71.9 (13.1)	74.6 (11.2)	0.59
CrCP Ipsilateral (mmHg)	45.8 (21.2)	48.1 (18.6)	0.78
CrCP Contralateral (mmHg)	44.8 (13.1)	45.2 (14.3)	0.95
RAP Ipsilateral (mmHg.s/cm)	1.3 (0.7)	1.1 (0.4)	0.56
RAP Contralateral (mmHg.s/cm)	1.2 (0.6)	1.1 (0.5)	0.92

486 Values are mean (SD). CBFV, cerebral blood velocity; BP, blood pressure; $EtCO_2$, end-tidal arterial
487 pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI,
488 Autoregulation Index.

489 **Table 3:** Haemodynamic characteristics at 10-14 day follow-up.

	Baseline	Hypocapnic Intervention	p-value
$EtCO_2$ (mmHg)	36.5 (4.9)	29.5 (7.3)	**0.04**
CBFV Ipsilateral ($cm.s^{-1}$)	41.4 (16.7)	39.5 (15.9)	0.82
CBFV Contralateral ($cm.s^{-1}$)	49.3 (22.3)	41.8 (15.7)	0.48
ARI Ipsilateral	4.7 (1.7)	5.4 (2.7)	0.56
ARI Contralateral	4.5 (1.9)	6.1 (2.0)	0.15
Mean BP (mmHg)	115.6 (19.5)	113.7 (12.3)	0.82
Heart Rate (bpm)	64.7 (10.3)	71.4 (8.0)	0.17
CrCP Ipsilateral (mmHg)	50.3 (27.5)	55.5 (28.2)	0.71
CrCP Contralateral (mmHg)	52.6 (13.1)	54.0 (23.4)	0.92
RAP Ipsilateral (mmHg.s/cm)	1.6 (0.5)	1.5 (0.3)	0.72
RAP Contralateral (mmHg.s/cm)	1.3 (0.5)	1.4 (0.4)	0.74

490 Values are mean (SD). CBFV, cerebral blood velocity; BP, blood pressure; $EtCO_2$, end-tidal arterial
491 pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI,
492 Autoregulation Index.

Figure Legend

Figure 1 A) CBFV ipsilateral, B) CBFV contralateral, C) ARI ipsilateral and D) ARI contralateral to end-tidal CO_2 change from baseline assessment to hypocapnia intervention. With the exception of patient #10, the lowest value of $EtCO_2$ corresponds to hyperventilation.

Figure 2 Delta (Δ) $EtCO_2$ change and corresponding delta (Δ) CBFV and ARI changes.

Figure 3 A) CBFV ipsilateral, B) CBFV contralateral, C) ARI ipsilateral and D) ARI contralateral to end-tidal CO_2 change from baseline assessment to hypocapnia intervention.