Effects of acetazolamide on the micro- and macro-vascular cerebral hemodynamics: a diffuse optical and transcranial doppler ultrasound study

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Abstract: Acetazolamide (ACZ) was used to stimulate the cerebral vasculature on ten healthy volunteers to assess the cerebral vasomotor reactivity (CVR). We have combined near infrared spectroscopy (NIRS), diffuse correlation spectroscopy (DCS) and transcranial Doppler (TCD) technologies to non-invasively assess CVR in real-time by measuring oxy- and deoxy-hemoglobin concentrations, using NIRS, local cerebral blood flow (CBF), using DCS, and blood flow velocity (CBFV) in the middle cerebral artery, using TCD. Robust and persistent increases in oxy-hemoglobin concentration, CBF and CBFV were observed. A significant agreement was found between macro-vascular (TCD) and micro-vascular (DCS) hemodynamics, between the NIRS and TCD data, and also within NIRS and DCS results. The relative cerebral metabolic rate of oxygen, $rCMRO_2$, was also determined, and no significant change was observed. Our results showed that the combined diffuse optics-ultrasound technique is viable to follow (CVR) and $rCMRO_2$ changes in adults, continuously, at the bed-side and in real time.

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OCIS codes: (170.3660) Light propagation in tissues; (170.3890) Medical optics instrumentation; (170.6480) Spectroscopy, speckle; (170.7170) Ultrasound; (290.4210) Multiple scattering.

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1. Introduction

The inherent ability of the cerebral vasculature to conserve constant cerebral blood flow (CBF) over a wide range of cerebral perfusion pressure (CPP) is known as the cerebral autoregulation (CAR) [1, 2]. One of the main mechanisms responsible for CAR is the ability of the cerebral arterioles to vasodilate/vasoconstrict in response to chemical and other stimuli. This is also known as the cerebral vasomotor reactivity (CVR), and is very important for the maintenance of healthy brain function through CAR and the general metabolic regulation of the brain tissue [3].

In the clinics and in research settings, CVR is assessed by the use of various stimuli such as carbon-dioxide (CO_2) inhalation [4], breath holding, hyperventilation and acetazolamide (ACZ) administration [5, 6]. The ACZ administration and CO_2 inhalation are the most frequently used of such stimuli. However, since CO_2 inhalation can cause discomfort due to gasping especially in non-cooperating patients, ACZ administration is often preferred to assess CVR [7]. An intravenous (i.v.) dose of 500 to 1000 mg of ACZ, in humans, dilates the arterioles globally without producing any (or minimal) change in the blood pressure. The dilation of the arterioles reduces the resistance against blood flow while CPP is preserved. Therefore, the CBF increases globally both in the micro-vasculature and the major cerebral arteries (macro-vasculature) [8–11]. At these doses, it is generally assumed that this corresponds to a measure of the maximal CVR and can be used to infer information about the vascular reserve.

To date, a variety of techniques were used to follow the effect of ACZ administration on CBF; single-photon-emission-computed-tomography (SPECT) [12, 13], flow-sensitive-alternating-inversion-recovery (FAIR) perfusion MRI [14], Continuous-arterial-spin-labeling (CASL) MRI [15], $H_2^{15}O$ Positron-emission-tomography (PET) [16] and transcranial Doppler (TCD) ultrasonography [17–19]. In general, these techniques are reliable but they are often expensive (SPECT, FAIR, CASL, PET), are limited to the macro-vasculature (TCD), require patient transport (SPECT, FAIR, CASL, PET) or injection of contrast agents (SPECT, PET). They are not, therefore, applicable to all patients. A non-invasive, bed-side technique that allows access to the micro-vascular and local CBF without any contrast agents and with relative ease of use and deployment is desirable.

Near-infrared spectroscopy (NIRS) or diffuse optical spectroscopy (DOS) is a non-invasive, portable method for continuous and bedside recording of cerebral, microvascular, blood oxygen saturation (StO_2) and the total hemoglobin concentration (THC) [20, 21]. The method is based on the fact that the near-infrared light (~ 650 – 950 nm) can penetrate several centimeters into deep tissues where it is predominantly absorbed by oxy- and deoxy-hemoglobin species [20, 21]. NIRS provides a direct measure of oxy- and deoxy-hemoglobin concentration as well as total hemoglobin concentration [THC, often proportional to the cerebral blood volume (CBV)] [20]. NIRS could also offer high temporal resolution ($\sim$ ms) to observe transient hemodynamic phenomena such those in response to functional stimuli. Due to these properties, NIRS has emerged as a promising bed-side monitor [21, 22].

In order to estimate CBF, NIRS looks at either the changes in CBV or uses exogenous tracers such as indo-cyanine green (ICG) [21, 22]. However, it is possible that CBF is disassociated from CBV in some pathological conditions. Moreover, the use of exogenous tracers for CBF assessment [23, 24] are prohibitively difficult in the case of transient measurements [25]. For example, Tachtsidis et. al. [26] and Schytz et. al. [6] have used ICG to measure CBF changes in response to ACZ administration and have reported that they were unable to obtain reliable CBF measurements. Therefore, NIRS has typically been used for the transcranial measurement of the cerebral blood oxygenation and is often accompanied with other modalities like PET, SPECT or TCD to measure CBF [13, 26–28].

In this study, we demonstrate successful measurements of CBF in response to ACZ administration by using a related technique, diffuse correlation spectroscopy (DCS) [or diffuse-wave-

spectroscopy (DWS)] [22, 29, 30]. DCS allows us to non-invasively and continuously measure micro-vascular, local CBF in deep tissues without any need of tracers. The technique has been validated *in vivo*, in tissues, against different standard methods for CBF measurement [22] such as Doppler ultrasonography, arterial-spin-labeled MRI, Xenon-CT, laser Doppler and against invasive and non-invasive measures of physiology. Currently, it is able to measure only the relative changes in CBF but recent reports suggest that with proper calibration and better physical modeling it has the potential for absolute measurements [22, 31, 32]. In adult human brain, DCS was first utilized to measure the hemodynamic response to functional stimuli [33, 34]. Later on, in clinical populations, DCS use was reported in premature born babies [31, 32], in neonates born with congenital heart defects [35], traumatic brain injury patients [36] and in acute ischemic stroke patients [37].

In most of these studies (including the present study), DCS is combined with NIRS so that the measures of microvascular CBF and oxy- and deoxy-hemoglobin concentrations could be employed together to assess the cerebral metabolic rate of oxygen extraction ($CMRO_2$). The measurement of $CMRO_2$, which is proportional to the rate at which oxygen is consumed in the brain by metabolic processes, is potentially of great importance for the clinical assessment of the functioning of the cerebral tissue, for the evaluation of the extent of cerebral injury due to cerebrovascular diseases and for predicting the tissue survival [38, 39]. Preliminary studies have shown that $CMRO_2$ could be a more useful clinical measure than CBF or cerebral tissue oxygenation alone [40].

Unfortunately, bed-side measurements of $CMRO_2$ are prohibitively difficult. Currently available modalities for $rCMRO_2$ assessment in clinical settings, PET, SPECT and MRI require patient transport, lengthy measurement times, costly equipment and use of ionizing radiation (in the case of PET and SPECT). In addition $rCMRO_2$ data, measured with these modalities are available only at limited time-points. Therefore, it is not surprising that the $rCMRO_2$ change upon ACZ administration is still being debated [5, 41, 42] with contradictory reports [7]. The diffuse optical methods that we employ are the current prominent promising modalities for bed-side use to measure the $rCMRO_2$.

The CVR and $CMRO_2$ measurements in response to ACZ administration may have a clinical significance for the management of patients with acute and/or chronic cerebrovascular diseases. For example, the CVR for patients with severe steno-occlusion of the carotid artery were intensively studied while there is a higher stroke risk for patients with steno-occlusive diseases who also suffer impaired CVR [43, 44]. Therefore, bed-side, continuous and real-time $rCMRO_2$ measure with the hybrid NIRS-DCS technique may help the clinicians to guide therapeutic strategies.

In the present work, we have applied a hybrid NIRS-DCS device to follow CBF, THC and $CMRO_2$ changes after ACZ bolus continuously on ten healthy subjects. Furthermore, we have utilized a clinical TCD instrument to measure the macro-vascular cerebral blood flow velocity (CBFV) in the middle-cerebral artery (MCA). This enabled us to measure the CVR for micro- and macro-vasculature simultaneously along with NIRS measures of micro-vascular oxy- and deoxy-hemoglobin concentrations. By acquiring data from two hemispheres, we were able to assess the hypothesis that the changes are similar and global in healthy subjects. To the best of our knowledge, this study is the first report of the comparison of DCS to TCD in adults for CVR assessment.

2. Materials and methods

2.1. Measurement protocol

The measurements were carried out at the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain where the study protocol was approved by the local ethics review board. Before the

Fig. 1. (a) The time line of the study protocol. (b) A schematic representation of the placement of optical and ultrasound probes.

measurement each subject underwent a general clinical assessment and a detailed survey was filled to verify that they were qualified as “healthy” subjects with no prior cerebrovascular problems. The subjects were placed in a supine position in a quiet room and venous catheter was inserted into the right antecubital vein for the drug injection.

Optodes (optical probes) fixed on black foam pads were placed symmetrically on the forehead, ~ 1 cm left and right of the mid-line, to avoid the sinuses [Fig. 1(b)]. The optodes were tightly attached onto the skin using medical tape and further by the head-frame used for placing the transcranial Doppler ultrasound probes.

Both left and right middle cerebral arteries MCA were insonated through the temporal acoustic window by an experienced neurologist. After placing the optodes and TCD probes and after optimizing the optical and ultrasound signals, five minutes of baseline data were obtained. ACZ (see below for details) was then injected for ~ 1 minute and the hemodynamics was followed concurrently throughout with both optical and ultrasound methods for another 20 min after ACZ infusion. All events were marked synchronously for both optics and ultrasound [Fig. 1(a)]. Heart rate and systolic, diastolic and mean blood pressures were measured before ACZ injection and at the last 2 minutes of the measurement with an auto-inflated blood pressure monitor (OMRON M6, HEM-7001-E).

2.2. Acetazolamide

Acetazolamide is a selective inhibitor of carbonic anhydrase (EC 4.2.1.1). Intravenous (i.v.) administration of a dose >10 mg/kg causes the inhibition of carbonic anhydrase activity in most tissues and increases the CBF by the dilation of the arterioles [41,45]. In this work, 1000mg Acetazolamide(ACZ) dissolved in 10ml of saline was given as a bolus through an i.v. catheter.

2.3. Optical method

We have utilized two instruments (NIRS and DCS) that were mounted together on a portable cart and were synchronized through custom software and hardware in a similar manner to previous studies [33,46]. The NIRS measurements were carried out in the frequency-domain (110MHz) with 690nm, 785nm and 830nm lasers and two PMTs for light detection (ISS model 95230 Imagent) [47]. As depicted in [Fig. 1(b)], a single source-detector separation (2.5cm) was

used for NIRS measurements for ease of use for the placement of the two probes on forehead (one for each hemisphere).

The successful use of a single-source detector separation frequency domain NIRS with DCS was reported in many of the pioneering studies [22, 33] but often with corrections for partial volume effects in adults [33]. Since we used a modified form of the Beer-Lambert law, the main concern here was to be able to accurately evaluate the effective optical pathlength [differential pathlength factor (DPF)] [48, 49]. This required us to consider partial volume effects due to the presence of superficial layers such as the skull and scalp tissues. To this end, we have used a correction factor, i.e. a partial pathlength factor (PPF) based on the previous studies [33, 50]. In particular, we have utilized the DPF values that were measured with a time-resolved NIRS in Ref. [51] (DPF=5.86 at 832nm and DPF=6.51 at 690nm averaged over 50 male and 50 female with median age of 33), and the PPF values for the same ACZ challenge, reported in Ref. [52] (PPF=0.44 for 2.5cm source-detector separation). We note that these corrections do not, in general, alter the trends but they affect the absolute values. The absolute values of the changes are also important when assessing the CMRO₂ changes since DCS may have a different partial volume effect (see below). We employed the *THC* results to derive an alternative CVR index according to the relation $CVR_{THC} = \frac{\Delta THC}{THC_{bl}} \times 100$. Throughout this paper, the subscript *bl* is to indicate the baseline values and Δ shows the changes from the baseline.

The CBF was assessed with DCS. Like NIRS, DCS also uses the biological window to penetrate in deep tissue but DCS uses intensity correlation of the scattered light to evaluate the motion of the scatterers, i.e. in this case red blood cells [22]. This intensity correlation data are then fitted using the solutions for the photon correlation diffusion equation in a semi-infinite geometry to extract the information about movement of the light scatterers in deep tissue (blood flow) [22]. The DCS measures are used to determine relative cerebral blood flow ($rCBF = \frac{\Delta CBF + CBF_{bl}}{CBF_{bl}}$) and further to derive a “standard” CVR according to $CVR_{DCS} = (rCBF - 1) \times 100$.

DCS device uses two long-coherence-length CW lasers at 785nm and eight avalanche-photodiode (APD) detectors whose output is fed to a custom-built hardware autocorrelator. In this measurement the same source-detector separation as NIRS was used for the DCS measurements in a “cross formation” [Fig. 1(b)] enabling the measurement of CBF at roughly the same tissue volume as the NIRS data. The equipment, the analysis method and the probe were described in details in our previous studies [22, 33, 37].

The optical data acquisition is interlaced between a 0.5 second of NIRS and 3 seconds of DCS measurements. Since no light leakage from source of one probe to the detector of the other is observed, NIRS and DCS measurements were carried out in both probes simultaneously to reduce the measurement time per data point leading to a measurement time of about 4 seconds.

The changes in *rCMRO₂* are calculated for each hemisphere separately using [53]:

$$rCMRO_2 = \left(\frac{\Delta CMRO_2 + CMRO_{2,bl}}{CMRO_{2,bl}} \right) = \left(1 + \gamma_r \frac{\Delta Hb}{Hb_{bl}} \right) \times \left(1 + \gamma_t \frac{\Delta THC}{THC_{bl}} \right)^{-1} \times (rCBF) \quad (1)$$

where $\gamma_r = \frac{\Delta Hb_V \cdot Hb_{bl}}{\Delta Hb \cdot Hb_{bl,V}}$ and $\gamma_t = \frac{\Delta THC_V \cdot THC_{bl}}{\Delta THC \cdot THC_{bl,V}}$ with Hb_V and THC_V being the baseline deoxy-hemoglobin and total hemoglobin concentrations in the venous compartment respectively and Hb and THC representing the same in tissue. Finally *rCBF* represents relative cerebral blood flow as defined above.

The *rCMRO₂* analysis is carried out by assuming $\gamma_t = \gamma_r = 1$ and baseline *THC* of 100 μ M and oxygen saturation level ($S_rO_2 = \frac{[HbO_2]_{bl}}{[HbO_2]_{bl} + [Hb]_{bl}} = 71\%$, $[HbO_2]_{bl}$ being the baseline oxy-hemoglobin concentration [54]). This implies 71 μ M for oxy-hemoglobin and 29 μ M for

deoxy-hemoglobin concentrations during the baseline period.

2.4. Transcranial Doppler ultrasound

CBFV recording of the MCA was obtained with a transcranial Doppler (TCD) instrument (DWL MultiDop-T digital, DWL Elektronische Systeme GmbH). Two probes are used in a range-gated and pulsed-wave mode at a frequency of 2 MHz. The probes were positioned over the temporal bone bilaterally using a standard head frame (Diamon®, DWL) that permitted a constant insonation angle. Simultaneous and continuous measurement of both MCA was performed at a depth of 50 to 60 mm (Fig. 1).

The TCD data were employed to calculate the relative cerebral blood flow velocity ($rCBFV = \frac{\Delta CBFV + CBFV_{bl}}{CBFV_{bl}}$) and later to calculate CVR values. The CVR values are calculated from TCD measures according to $CVR_{TCD} = (rCBFV - 1) \times 100$. Moreover, we have replaced the rCBFV measures with the rCBF data in Eq. (1) in order to re-calculate the $rCMRO_2$ values based on NIRS and TCD measurements.

2.5. Statistical analysis

NIRS, DCS and TCD data are stored as continuous variables over time. The data for the last 2 minutes of the measurement are then averaged to obtain the relative change from averaged 5 minute baseline (i.e. before injection) data. All values are reported as mean $\pm$ standard-deviation (STD) and are used for further calculations. The two sided Wilcoxon rank sum test with 95% confidence level is used to compare the data from left and right hemispheres as well as the DCS and TCD data with the null hypothesis that the distribution of two samples does not differ. The Wilcoxon-Mann-Whitney test (U-test) is used to evaluate the effect of the drug (ACZ) on the NIRS, DCS and TCD parameters (R project [55]). Pearson-product-moment-correlation-test is used to test for the correlation within and in-between optical and ultrasound modalities [55].

3. Results

We have recruited ten healthy volunteers (8 males and 2 females), age 30.6 ± 8 years (mean $\pm$ STD). Systolic and diastolic mean-arterial-blood-pressure together with heart rate are measured at the beginning (during 5 minutes baseline measurement) and at the last 2 minutes of the measurement with the blood pressure monitor. No change is observed upon ACZ administration among the studied subjects, in accordance with [11]. All ten subjects completed the study without any adverse affects. However, TCD data on one subject was missing because of technical issues.

Figures 2(a), 2(b), and 2(c) show the temporal evolution of the micro- and macro-vascular hemodynamics, averaged for all studied subjects. Figures 3(a), 3(b), and 3(c) show box-plots of the population data separately for right and left hemispheres and the overall results is summarized in Table 1. We now outline the results from different parameters in details.

Figure 2(a) shows the temporal evolution of the NIRS results averaged over the whole population. After ACZ administration, as expected, the oxy-hemoglobin concentration increased and deoxy-hemoglobin concentration decreased slightly. The resulting population data is further summarized in Fig. 3(a). For the whole population, the NIRS results for right and left hemispheres did not differ significantly ($p=0.24$ for oxy-hemoglobin and $p=0.1$ for deoxy-hemoglobin). Therefore, the right and left hemisphere data were averaged for the rest of the calculations which indicate that the oxy-hemoglobin increased by $10.1 \pm 6.6 \mu M$ and the deoxy-hemoglobin decreased by $1 \pm 1.6 \mu M$, after the ACZ administration compared to the baseline. However, while we have observed a significant increase in the oxy-hemoglobin concentration after ACZ administration ($p=0.0039$), the decrease of deoxy-hemoglobin was not statistically

significant ($p=0.13$). This was most probably due to the higher, relative standard-deviation of deoxy-hemoglobin measurement ($\frac{STD}{mean}(\%) = 153$). If we further use the assumed baseline values (see Section 2.3) then we obtain a 14.3% increase for oxy-hemoglobin and 3.6% decrease for deoxy-hemoglobin concentration changes.

$rCBFV$ and DCS $rCBF$ changes due to ACZ infusion are shown in Fig. 2(b) for all subjects. As seen in Fig. 2(b), as expected, both $rCBF$ and $rCBFV$ values increase after ACZ administration. The $rCBF$ and $rCBFV$ values for the whole population from the right and left hemispheres is summarized in Fig. 3(b). Similar to NIRS data, since the right and left hemisphere data for DCS ($p=0.97$) and for TCD ($p=1.0$) do not differ significantly, the right and left data for DCS and for TCD are averaged for subsequent calculations. $rCBF$ measured by DCS and $rCBFV$ measured by TCD data are in good agreement ($p=0.27$). We note that since the changes in CBF and CBFV were relatively tight across the whole population, i.e. the spread across subjects was to the same order as individual standard-deviations, we did not observe a correlation ($r=-0.05$, $p=0.9$) between two modalities but, as reported above, we have observed a good agreement.

The NIRS data was also compared to TCD results. We did not find a significant correlation of $rCBFV$ measures with either oxy-hemoglobin ($r=0.307$, $p=0.422$) or with deoxy-hemoglobin concentration changes ($r=0.109$, $p=0.78$).

We have also compared the two optical modalities. We have found a strong correlation between $rCBF$ measures with both oxy-hemoglobin concentration data ($r=0.716$, $p=0.0198$) and deoxy-hemoglobin concentration changes ($r=-0.9$, $P=0.0004$). The negative correlation between deoxy-hemoglobin and $rCBF$ is because $rCBF$ and deoxy-hemoglobin concentration changes after ACZ are in opposite directions. Overall, this is expected since in this particular challenge where the hemodynamics are driven by a series of biochemical events that lead to the dilation of the arterioles with minimal (see below) alterations in oxygen metabolism, increased CBF leads to increased blood oxygenation in a proportional manner.

CVR indices were calculated from $rCBF$, $rCBFV$ and NIRS data as described in Sections 2.3 and 2.4. They show $29 \pm 17.5\%$ (Mean $\pm$ STD) increase according to DCS measures, $36.9 \pm 10.8\%$ increase according to TCD and $9.1 \pm 5.6\%$ increase according to NIRS after ACZ administration.

Furthermore, we have also calculated the so-called ‘‘Grubb exponent’’ [56] which is the ratio of THC changes to CBF changes using a logarithmic formulation [57]. The Grubb exponent was fitted to be 0.23 for our test which is in reasonable agreement with the literature.

The $rCMRO_2$ changes were calculated point-by-point from NIRS and DCS data as described in Section 2.3. The average $rCMRO_2$ change with time over all studied subjects is depicted in Fig. 2(c). Interestingly, $rCMRO_2$ shows an initial increase which partly recovers to the baseline towards the end of the study which corresponds to the period that is averaged for all the measurements. Note, however, that the changes are small compared to the $rCBF$ and $rCBFV$ values. Similar to NIRS and DCS data, $rCMRO_2$ values for right and left hemispheres are also statistically identical ($p=0.064$). Figure 3(c) shows the box-plot of $rCMRO_2$ changes for the whole population compared to the baseline after ACZ administration. For the overall population, the change observed for $rCMRO_2$ ($9.9 \pm 15\%$) after ACZ administration is not significant ($p=0.131$), i.e. $CMRO_2$ calculated from NIRS and DCS data is unchanged after ACZ administration.

In order to compare local *micro-vascular* CBF and *macro-vascular* CBFV, $rCMRO_2$ was also calculated point-by-point using NIRS and TCD data as described in Section 2.4 (data are not shown). As for $rCMRO_2$ values calculated from NIRS and DCS measures, the right and left hemispheres are statistically same in the case of $rCMRO_2$ data obtained from NIRS and TCD measurements ($p=0.258$). However, the $rCMRO_2$ values from NIRS and TCD data show a significant change upon ACZ infusion ($p=0.0039$) with $23.1 \pm 13.1\%$ (mean $\pm$ STD), i.e.

Fig. 2. Average data for all studied subjects (8 male, 2 female, mean age 30.6) showing (a) the oxy- and deoxy-hemoglobin changes due to ACZ administration (NIRS data);(b) hemodynamic response to ACZ observed by changes in CBF measured by DCS and CBFV measured by TCD; and (c) changes in $rCMRO_2$ calculated point-by-point from changes in oxy- and deoxy-hemoglobin and rCBF. Vertical bold lines show the start of the baseline, start of injection, end of injection, and end of the measurement respectively. The data between the vertical dashed line and the end of the measurement have been averaged and used for further analysis. The error bars show the standard-deviation values at about 1.5 minute intervals.

Fig. 3. Box-plots summarizing the changes of: (a) oxy- and deoxy-hemoglobin concentrations; (b) rCBF (DCS) and rCBFV (TCD); and (c) $rCMRO_2$, compared to the baseline after ACZ injection. Here, L:left and R:right.

unlike the data from the micro-vasculature measured by DCS, $rCMRO_2$ calculated from NIRS and TCD data shows a significant increase upon ACZ infusion.

Table 1. Mean ($\pm$ standard-deviation, STD) and p-values for optical and ultrasound data. Superscript, *, implies that observed change is statistically significant ($p < 0.05$)

Case	Mean	STD	p-value
ΔHbO_2 (μM)*	10.1	6.6	0.0039
ΔHb (μM)	-1	1.6	0.131
$\Delta rCBF_{DCS}^*$ (%)	29	17.5	0.0019
$\Delta rCBFV_{TCD}^*$ (%)	36.9	10.8	0.0078
$\Delta rCMRO_2$ (%)	9.9	15	0.131

4. Discussion

We have studied the effects of ACZ administration on cerebral hemodynamics from both macro-vascular (using TCD) and micro-vascular (using diffuse optics) perspectives. Furthermore, by combining two diffuse optical techniques, NIRS and DCS, we were able to deduce information about changes in $CMRO_2$. We now discuss our findings, compare them to the literature, highlight its strengths and weaknesses, and finally suggest some further clinical applications.

ACZ caused (see Section 3) robust, persistent increases in oxy-hemoglobin concentration, CBF and CBFV in accordance with other reports on ACZ effect [6, 7, 41, 52]. These changes are known to be due to the dilation of the micro-vasculature in response to ACZ stimulus. In addition, since ACZ is injected in the blood stream, the changes observed in the brain in healthy people are global [7, 52]. This justified our use of the forehead, which is easily accessible, for measuring the micro-vascular hemodynamics in the frontal lobes as a measure CVR for a given hemisphere. We have confirmed this by observing statistically same changes for the left and right hemispheres [Figs. 3(a) and 3(b)].

According to our results oxy-hemoglobin concentration increased by 14.3% of its baseline value. The deoxy-hemoglobin decreased by 3.6% of its baseline value on average, leading to a 10.7% THC increase after ACZ administration. However the measured changes for deoxy-hemoglobin concentration were not statistically significant ($p=0.13$, Wilcoxon-Mann-Whitney test). This was most probably due to a higher, relative standard deviation of deoxy-hemoglobin concentration changes. Never the less, our values for mean THC changes after ACZ injection are in good agreement with the data reported elsewhere with continuous-wave-NIRS (THC increased by 5.7%) [13], time-resolved-NIRS (THC increased by 10.6%) [52] and ^{99m}Tc SPECT (THC increased by 9.4%) [12].

Since a single source-detector separation was used for the NIRS measurements, we have only measured the changes of oxy- and deoxy-hemoglobin concentrations, compared to their values before ACZ administration and not the absolute values. Furthermore, as detailed in Section 2.3, we have utilized literature values for the assumed optical pathlengths (DPF) with corrections for the partial volume effects (PPF). This may have led to some errors in our data due to inverse dependence of the reported oxy- and deoxy- hemoglobin concentrations on the assumed DPF and PPF values [48, 49]. However we note, that the subjects were in good health, and that we have considered their mean age, gender and the laser wavelength when using the DPF values from the literature [51]. Furthermore, the PPF values in the literature [52] were actually measured using the same ACZ challenge. The standard-deviation of the values from these reports gives us an idea of the errors that could be introduced to our results and since they are within the standard-deviation of our NIRS data, we expect that our errors are minimal. A future study

with a calibrated frequency domain device or a time-resolved instrument is planned.

The main impact of this work is to introduce the DCS technique to measure local changes of CBF in the micro-vasculature to follow the ACZ effect on CBF. DCS continuously measures local rCBF directly, without any further need to utilize isotope or non-isotope tracers. Our study shows 29% increase in the rCBF after ACZ bolus in healthy subjects, which is in good agreement with several studies (32.5% increase of rCBF on average) [14,58,59]. The reported values for the CBF change due to ACZ, range from 25% using ^{133}Xe SPECT [60] to 70% using ^{133}Xe [5] inhalation techniques. These results partly depend on the measurement technique. They also depend on the age and gender of the studied population.

TCD is the primary modality used for CVR assessment, which is mainly due to its accessibility and simplicity compared to the other relevant techniques. Both our work and the others [18,58,59,61,62] suggest that an intravenous administration of ACZ induces a significant increase in rCBFV. We have found a 37% increase of rCBFV in MCA after ACZ bolus, which is in good agreement with the results reported in the above cited studies.

The CVR values were also calculated from THC data and led to $9.1 \pm 5.6\%$ increase after ACZ injection. It is three and four times smaller than the CVR values calculated from DCS and TCD data, respectively. This deviation is expected since the NIRS measured THC changes do not directly correspond to the blood flow changes [25]. However, if we use the Grubb's formulation [56], with the Grubb exponent that we have measured ($G=0.23$ for DCS/NIRS [57]), we can correct for this under-estimation. We note that the calculation of the exponent depends on the assumed baseline value for THC.

Furthermore, we have, to the best of our knowledge, reported the first comparison of DCS measures of local microvascular CBF to TCD measures of middle-cerebral artery CBFV in adults. A comparison between micro- and macro- vasculature response with a similar approach (TCD-DCS) was previously reported in premature neonates by us [63] and others [64]. In both studies, the authors looked at the absolute, baseline values of CBFV and CBF in premature born infants and reported significant correlations between TCD and DCS results. In an earlier paper [63], it was attempted to compare changes due to head-of-bed challenge in the premature born infants. There a non-significant change was induced, which was observed by both TCD and DCS. Since the partial volume effects are often negligible in premature born infants with their thin skulls and scalps, it was not obvious whether a correlation would be observed in the adult brain. Furthermore, we also note that, in the same work, R-Labarbe et. al. [64] demonstrated that the use of DCS and NIRS simultaneously has provided better estimates of CMRO_2 than NIRS only measures.

One immediate consequence of having concurrent measures of blood flow in micro- and macro-vasculature is the capability of comparing their interaction mechanism. This question was indirectly addressed during the early works carried out to validate TCD against standard ^{133}Xe inhalation SPECT for CBF measurements [58,59,65]. They have found a significant correlation between CBFV values (measured with TCD) and CBF values in the same artery (measured with SPECT) [59] or CBFV values in the artery with local CBF values in the territory of that same artery (measured with SPECT) [58,65]. However the prediction of the local CBF on the basis of CBFV data is only valid if among other factors the diameter of the large vessels remain unchanged [66]. A $> 4\%$ increase in the diameter of the MCA upon ACZ administration was reported [18], which may hinder this comparison. Nevertheless, our results showed that the local blood flow change in micro-vasculature, rCBF, and the blood flow velocity change in the macro-vasculature, rCBFV, are in good agreement for healthy subjects ($p=0.27$). The advantages posed by DCS that we have discussed earlier would enable large scale studies on the interactions between micro- and macro-vasculature.

Our results demonstrate the capability of the hybrid NIRS-DCS technique, to give informa-

tion about the dynamics of CBF change in addition to that of the oxy-hemoglobin and deoxy-hemoglobin concentration changes in response to ACZ challenge. According to Figs. 2(a) and 2(b), upon ACZ administration oxy- hemoglobin concentration and rCBF increases along with decrease in the deoxy-hemoglobin concentration. Moreover, the optical data change continuously until the end of measurement. However it should be mentioned that, these figures show the average trend over all studied subjects and the dynamics of the changes varies amongst the individual subjects. In order to interpret the dynamics of the observed changes correctly, we would have to consider variations in the subject physiology including the weight, age and gender with continuous measures of the systemic physiology such as the blood pressures and the arterial pressure of carbon-dioxide in a larger population which is beyond the aim of this manuscript.

One important factor that could affect the optical measurements, NIRS and DCS, is the change in superficial tissue oxygenation and blood flow after ACZ bolus especially with a single source-detector separation which does not allow us to correct for those effects. These changes, if they occur, would be added to the changes of cerebral tissue oxygenation and blood flow leading to the misinterpretation of the results from the cerebral tissue. The changes in superficial tissue oxygenation and blood flow due to ACZ bolus was studied by Tachtsidis et. al. [26], by CW-NIRS with different source-detector separations, and by Kohri et. al. [52] by time-resolved-NIRS. They did not observe any changes after ACZ bolus on superficial tissue oxygenation. Furthermore, by utilizing a laser Doppler probe, Tachtsidis et. al. [26] have confirmed that the blood flow does not also change in the superficial tissues. Based on these findings, we have assumed that the superficial tissue oxygenation and blood flow are unchanged after ACZ injection.

Concurrent measures of oxy-hemoglobin concentration, deoxy- hemoglobin concentration and rCBF are further used for $rCMRO_2$ calculation. The study of the ACZ effect on $rCMRO_2$ is important for both clinical and methodological reasons. Since ACZ is used as a treatment for some diseases in addition to its use as a diagnostic tool for CBF studies, the mechanism behind its CBF effect is of major physiological interest [5]. Moreover some investigators have used the difference in arterio-venous oxygen content ($(a-v)O_2$) to estimate changes in CBF after ACZ bolus. This would only be applicable if the $rCMRO_2$ does not change after ACZ administration. Although today it is mostly agreed that $rCMRO_2$ does not change after ACZ [5,41,62] but there are some contradictory reports [42].

Based on our NIRS and DCS data, we have calculated $rCMRO_2$ point-by-point over time [Fig. 2(c)], as described in Section 2.3. Overall, our $rCMRO_2$ data calculated from NIRS and DCS measures did not show a significant change after ACZ administration ($p=0.13$). We note that we have a rather high standard-deviation for $rCMRO_2$ data ($\frac{STD}{mean}(\%) = 152$), which is mostly due to high relative standard-deviation values in deoxy-hemoglobin concentration measurement ($\frac{STD}{mean}(\%) = 153$).

The $rCMRO_2$ values calculated point-by-point from NIRS and TCD data, on the other hand, have shown a significant increase after ACZ infusion ($p=0.0039$). Since both calculations share the same NIRS measures, according to Eq. (1) the only difference in the $rCMRO_2$ measures would be due to the fact that DCS measures micro-vascular blood flow while TCD gives the blood flow velocity in the macro-vasculature. Hence, due to slightly higher (yet statistically non-significant) values for blood flow velocity from TCD as compared to DCS, an increased $rCMRO_2$ is obtained. We believe that the $rCMRO_2$ values from NIRS and DCS data is more reliable and more relevant since they reflect local, micro-vascular physiology.

As the NIRS data are used for the $rCMRO_2$ calculation, all the corrections and baseline approximations that we have used to extract NIRS values affect the $rCMRO_2$ values. We have also assumed that the deoxy-hemoglobin concentration and THC changes in the venous, and in

the capillary compartments do not change after ACZ, i.e that $\gamma_r = \gamma_t = 1$ in Eq. (1) [67–69]. In order to test the effects of the assumed baseline values, we have varied the assumed baseline S_tO_2 and THC values and observed only minor effects on our $rCMRO_2$ measures. For example, a 10% change in THC caused less than 0.5% change in $rCMRO_2$ value and a 10% change in S_tO_2 value caused 1.5% change in $rCMRO_2$.

We note that the use of small and relatively simple probes with single source-detector separation makes practical for use in critically ill patients specially in the intensive care units. Since one of our goals is to utilize this data as a “control” group for future studies with carotid artery diseases and ambulatory stroke patients, this consideration was important. Besides, on those patients, if they have a unilateral problem, one hemisphere could be used as a control as in our previous study utilizing a head-of-bed challenge [37].

Moreover, it should be kept in mind that these results are very sensitive to rCBF changes, which may be the primary main reason for contradictory values reported in the literature for $rCMRO_2$. This could also immediately seen based on our $rCMRO_2$ calculations with shared NIRS but different blood flow data. We did not make any partial volume corrections to our CBF data obtained by DCS. Partial volume corrections for DCS were proposed in several studies in the past for local changes [33,34] and for layered tissues [70,71]. While this was expected, surprisingly, many other studies using stimuli that lead to global changes, as in our case, have reported good agreement between DCS and “gold standard” techniques such as Xenon-inhalation CT *without* any or minimal partial volume corrections [22, 35, 37, 63, 64, 72]. Overall, these results have shown that due to subtle differences between DCS and NIRS, the partial volume corrections (if any) are not straightforward and further research is needed. However, since the overwhelming evidence including the excellent agreement we have observed between TCD and DCS indicate that the corrections can be minimal, we have chosen not to implement them.

The CVR assessment is of great prognostic significance for patients with severe stenosis or occlusion of the large cerebral arteries. This is due to a higher stroke risk for patients with steno-occlusive diseases who also suffer decreased or exhausted CVR [43,44]. While yearly incidence of stroke between 2 and 8 % is reported for patients with steno-occlusive carotid diseases globally [73, 74], patients with impaired CVR have higher risk of stroke, between 27 to 55% [44, 75]. The CVR test with ACZ may then be of a significant value for identifying this high-risk sub-group of patients. It might also help clinicians to guide therapeutic interventions. A follow-up study is currently underway to assess the CVR in patients with steno-occlusive carotid diseases and to study CVR asymmetry in symptomatic and asymptomatic cases. Although similar questions have been addressed before with other modalities, our impact with diffuse optic-ultrasound techniques is continuous, bed-side measure of CVR both in micro- and macro-vasculature together with local measures of cerebral tissue oxygenation and oxygen metabolic rate. In addition it would reduce the complications due to patient transfer and use of ionizing tracers for CVR measurements.

5. Conclusion

In this study, we have demonstrated that the combined NIRS, DCS and TCD technique could be applied for the assessment of CVR upon ACZ administration. This is the first report where DCS is compared to TCD in adults. This combination of three portable, continuous and bed-side measures are advantageous over the other, mainly imaging, modalities used for similar studies where both the micro- and macro-vascular cerebral hemodynamics are studied. A key advantage of this approach is that it allows us to measure at the bed-side and without any need to utilize any tracer. We have demonstrated that macro- and micro-vascular blood flow and velocity changed in a similar manner and globally. The combination of DCS and NIRS enabled us to estimate changes in oxy- and deoxy-hemoglobin concentrations hence deriving a measure

of the cerebral oxygen metabolism ($rCMRO_2$). Since there is no need for patient transport, the set-up time is relatively short and there are no potential risks such as those due to the use of ionizing radioactivity, our approach is suitable for clinical settings such as the neuro-intensive care units.

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