

Chasing the evidence: the influence of data segmentation on estimates of
dynamic cerebral autoregulation

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Abstract

Objective: Transfer function analysis (TFA) of dynamic cerebral autoregulation (dCA) requires smoothing of spectral estimates using segmentation of the data (S_D). Systematic studies are required to elucidate the potential influence of S_D on dCA parameters.

Approach: Healthy subjects (HS, $n=237$) and acute ischaemic stroke patients (AIS, $n=98$) were included. Cerebral blood flow velocity (CBFV, transcranial Doppler ultrasound) was recorded supine at rest with continuous arterial blood pressure (BP, Finometer) for a minimum of five minutes. TFA was performed with durations $S_D = 100, 50$ or 25 s and 50% superposition to derive estimates of coherence, gain and phase for the BP-CBFV relationship. The autoregulation index (ARI) was estimated from the CBFV step response. Intrasubject reproducibility was expressed by the intraclass correlation coefficient (ICC).

Main results: In HS, the ARI, coherence, gain, and phase (low frequency) were influenced by S_D , but in AIS, phase (very low frequency) and ARI were not affected. ICC was excellent (>0.75) for all parameters, for both HS and AIS. For $S_D=100$ s, ARI was different between HS and AIS (mean $\pm$ sdev: 5.70 ± 1.61 vs 5.1 ± 2.0 ; $p<0.01$) and the significance of this difference was maintained for $S_D = 50$ s and 25 s. Using $S_D = 100$ s as reference, the rate of misclassification, based on a threshold of $ARI \leq 4$, was 6.3% for $S_D = 50$ s and 8.1% for $S_D = 25$ s in HS, with corresponding values of 11.7% and 8.2% in AIS patients, respectively.

Significance: Further studies are warranted with S_D values lower than the recommended standard of $S_D=100$ s, to explore possibilities of improving the reproducibility, sensitivity and prognostic value of TFA parameters used as metrics of dCA.

Keywords: cerebral blood flow, transcranial Doppler ultrasound, cerebral haemodynamics, transfer function analysis, reproducibility

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3 **1. Introduction**
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7 Dynamic cerebral autoregulation (dCA) expresses the transient response of cerebral blood flow (CBF)
8 to sudden changes in arterial blood pressure (BP). Although initially assessed as the response of CBF
9 velocity (CBFV), as measured with transcranial Doppler ultrasound (TCD), to a rapid drop in BP,
10 induced by the sudden released of inflated thigh cuffs (Aaslid et al. 1989), subsequent studies have
11 demonstrated that dCA can be assessed from spontaneous fluctuations in BP, by means of time- or
12 frequency-domain analyses (Panerai et al. 1996; Simpson et al. 2001; Zhang et al. 1998). By viewing
13 changes in BP as the input and corresponding changes in CBF (or CBFV) as the output, transfer
14 function analysis (TFA) is particularly suited to characterise the effectiveness of dCA by the
15 amplitude and frequency dependences of the gain and phase frequency responses (Panerai et al. 1996;
16 Zhang et al. 1998). As an adjunct, the coherence function can provide statistically objective criteria
17 for assessing the reliability of TFA parameters (Benignus 1969; Claassen et al. 2016; Panerai et al.
18 2006; Panerai et al. 2016), but has also been proposed as a metric of dCA efficiency (Giller 1990).
19
20 Early studies of dCA based on TFA were characterised by considerable diversity of the parameter
21 settings used to derive estimates of the auto- and cross-spectra required for calculation of gain, phase
22 and coherence (Meel-van den Abeelen et al. 2014a; Meel-van den Abeelen et al. 2014b). More
23 recently, a White Paper from the International Cerebral Autoregulation Research Network (CARNet)
24 has proposed more strict guidelines that should improve standardisation between studies and centres,
25 with potential benefits for comparability and robustness of clinical studies (Claassen et al. 2016).
26 Although the White Paper aimed to substantiate recommendations based on robust evidence, this was
27 not possible in many instances due to the lack of suitable studies in the literature (Claassen et al.
28 2016). One such aspect, that could be critical, is the duration of signal segmentation (S_D) that should
29 be adopted in conjunction with Welch’s method for smoothing spectral estimates, which is a standard
30 procedure in TFA (Bendat and Piersol 1986). For this purpose, the White Paper’s recommendation is
31 the use of recordings of BP and CBF(V) lasting at least 5 min, with data segmentation using window
32 durations of around 100 s, and 50% superposition (Bendat and Piersol 1986; Claassen et al. 2016).
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These recommendations were based on the prevalence of these settings in the literature (Meel-van den Abeelen et al. 2014b), but no objective evidence was provided to support these choices. Assuming that recordings with 5 min duration are usually adopted for most studies, a total of five segments with $S_D=100$ s can be extracted by using 50% superposition of segments. However, by reducing the duration of segments to $S_D= 50$ s, it should be possible to perform spectral smoothing with 11 segments, and this number could be extended to 23 if $S_D=25$ s. These different settings involve a trade-off between frequency resolution on one hand, and greater smoothing on the other. Shorter segments might also restrict the amount of low-frequency power that is included in spectral estimates, which should also be a consideration. To address these uncertainties, and to provide more definitive evidence about which choices should be recommended for greater standardisation of TFA applications to dCA assessment, we performed a sensitivity analysis of the effects of S_D on dCA metrics based on a large number of recordings from both healthy subjects (HS) and patients with acute ischaemic stroke (AIS). In summary, we tested the hypothesis that different values of S_D would not have a significant effect on estimates of gain, phase and coherence, as well as the autoregulation index (ARI) that can also be derived by means of TFA (Panerai et al. 1998b; Tiecks et al. 1995).

2. Methods

2.1 Study participants

Data for this analysis were obtained from several previous studies performed in HS and AIS patients and stored in the Leicester Cerebral Haemodynamics Database (Patel et al. 2016). All studies in the database had similar inclusion and exclusion criteria. Healthy subjects were 18 years of age or older, without any history or symptoms of cardiovascular, neurological or respiratory disease. AIS patients had diagnosis confirmed by neuroimaging and were admitted to the University Hospitals of Leicester NHS Trust, within 48 h with mild to moderate severity strokes as defined by a NIHSS (National Institute of Health Stroke Scale) ranging from 0 to 15. AIS patients were excluded if they had any previous history of myocardial infarction, respiratory disease, renal disease, or atrial fibrillation. A

total of 237 healthy subjects met these criteria and were included for further analysis. The corresponding number of stroke patients included was 98. Fifteen healthy subjects and 13 stroke patients were later removed due to technical criteria for acceptance of ARI estimates as described below. All studies contained in the database had local research Ethics Committee approval (Intharakham et al. 2019b; Patel et al. 2016), and all participants provided written informed consent.

2.2 Physiological measurements

Volunteers avoided caffeine, alcohol, and nicotine for ≥ 4 h before attending a research laboratory with controlled temperature (20-23°C) and free from visual or auditory stimulation. During the entire procedure, subjects were in a supine position and detailed instructions were given before taking measurements. Bilateral insonation of the middle cerebral arteries (MCAs) was performed using TCD (Viasys Companion III; Viasys Healthcare) with 2 MHz probes, which were secured in place using a head-frame. A 3-lead electrocardiogram (ECG) was recorded and end-tidal CO₂ (EtCO₂) was measured via nasal prongs (Salter Labs) by a capnograph (Capnograph Plus). BP was recorded continuously using the Finapres or Finometer devices (FMS, Finapres Measurement Systems, Arnhem, Netherlands), attached to the middle finger of the dominant hand of healthy subjects or the non-paretic hand of AIS patients. Both devices use the principle of arterial volume clamping of the digital artery and are considered interchangeable. The servo-correcting mechanism of the Finapres/Finometer was switched on and then off prior to measurements. Participants' systolic and diastolic BP were measured by classical brachial sphygmomanometry followed by a period of 15-min stabilisation and a 5-min baseline recording.

Data were simultaneously recorded onto a data acquisition system (PHYSIDAS, Department of Medical Physics, University Hospitals of Leicester) for subsequent off-line analysis using a sampling rate of 500 samples/s.

2.3 Data editing and analysis

All signals were visually inspected to identify artefacts; noise and narrow spikes (<100 ms) were removed by linear interpolation. CBFV channels were subjected to a median filter and all signals were low-pass filtered with a 8th order Butterworth filter with cut-off frequency of 20 Hz. BP was calibrated at the start of each recording using systolic and diastolic values obtained with sphygmomanometry. The R–R interval was then automatically marked from the ECG and beat-to-beat heart rate (HR) was plotted against time. Occasional missed marks caused spikes in the HR signal; these were manually removed by remarking the R–R intervals for the time points at which they occurred. Mean, systolic and diastolic BP and CBFV values were calculated for each cardiac cycle. The end of each expiratory phase was detected in the EtCO₂ signal, linearly interpolated, and resampled with each cardiac cycle. Beat-to-beat data were spline interpolated and resampled at 5 samples/s to produce signals with a uniform time-base.

TFA of the BP-CBFV relationship was performed using Welch's method using in-house software implemented in Fortran. The 5-min recordings were broken down into segments with S_D of 102.4, 51.2 or 25.6 s, respectively. With a sampling rate of 5 samples/s, these durations corresponded to N_W = 512, 256 or 128 samples, respectively. For simplicity, values of S_D will be referred to as 100, 50 and 25 s, respectively, in what follows. The mean values of BP and CBFV were removed from each segment and a cosine window was applied to minimise spectral leakage. With 50% superposition of segments, the number of segments (N_{SEG}) used to obtain estimates of the BP and CBFV auto- and cross-spectra were five, 11 and 23, respectively, for S_D values of 100, 50 and 25 s. The coherence function, amplitude (gain) and phase frequency responses were calculated from the smoothed auto- and cross-spectra using standard procedures (Claassen et al. 2016; Panerai et al. 1998a). The CBFV step response to the BP input was estimated using the inverse fast Fourier transform of gain and phase (Bendat and Piersol 1986).

The autoregulation index (ARI), which represents dynamic CA, was extracted by using the normalised minimum square error (NMSE) fit between the CBFV step response and one of the 10 model ARI curves proposed by Tiecks et al. (1995). ARI values were only accepted if the mean coherence function for the 0.15-0.25 Hz frequency interval was above its 95% confidence limit,

adjusted for the corresponding degrees of freedom, and the NMSE was ≤ 0.30 (Panerai et al. 2016). As detailed below, 95% confidence limits for coherence were calculated for each value of S_D as a function of the number of segments (Fig. 1).

2.4 Statistical analysis

Based on the sample sizes for both HC and AIS, data were treated as normally distributed after visual inspection of histograms and probability plots. Differences between parameters were assessed using the Student's *t*-test. Multiple parameter comparisons were performed with parametric repeated-measures ANOVA. Differences between values derived for the right and left hemispheres were averaged when no significant differences were found. In AIS patients, only the affected side was analysed as discussed below. To perform statistical tests on spectral parameters, values of coherence, gain and phase were averaged for the very low (VLF, 0.02-0.07 Hz) and low (LF, 0.07-0.20 Hz) frequency ranges (Claassen et al. 2016). In abbreviated form, Coh^{50}_{VLF} refers to the average coherence in the VLF interval for $S_D=50$ s, with similar format used to indicate mean values of gain and phase. A *p*-value of < 0.05 was assumed to indicate statistical significance.

Previous estimates of the 95% confidence limit of the mean coherence function, in the 0.15-0.25 Hz frequency interval (Panerai et al. 2016), were extended using the three different settings for S_D , to cover the entire range of N_{SEG} values, using the Monte-Carlo method previously described (Claassen et al. 2016; Panerai et al. 2016). Confidence limit values for discrete settings of N_{SEG} were interpolated with second or third order polynomials to provide an easier way to account for the contribution of degrees of freedom in further analyses (Fig. 1).

Intrasubject parameter changes due to different values of S_D were expressed with Bland-Altman plots (Bland and Altman 1986) and also with the intraclass correlation coefficient (ICC), that was classified as *poor* (<0.40), *fair* (0.40-0.59), *good* (0.60-0.74) or *excellent* (0.75-1.0) (Cicchetti 1994). A value of $ARI < 4$ was adopted as the criterion for impaired dCA (Caldas et al. 2017; Patel et al. 2016).

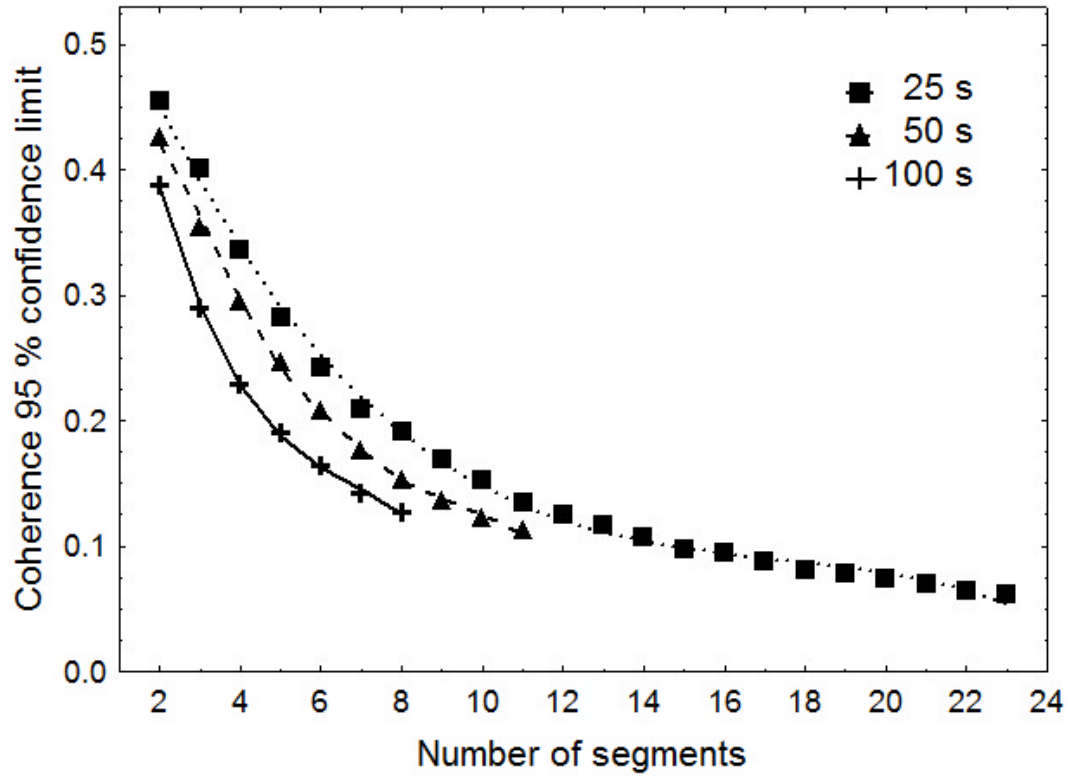


Figure 1 – Mean coherence 95 % confidence limits for the 0.15-0.25 Hz frequency interval for segment durations of 100 s (crosses, continuous line), 50 s (triangles, dashed line) and 25 s (squares, dotted lines) as a function of the total number of segments. For a 5 min recording, with 50% superposition, the maximum number of segments in each case are $N_{SEG} = 5$ (100 s), $N_{SEG} = 11$ (50 s) and $N_{SEG} = 23$ (25 s), respectively. Continuous lines are polynomial interpolations for the discrete values of N_{SEG} represented by symbols.

Results

HS and AIS patients included in the study correspond to all supine baseline recordings available in the database with duration ≥ 5 min, with good quality bilateral measurements of CBFV, totalling $n=237$ and $n=98$ participants, respectively. Table 1 provides additional demographic and physiological baseline characteristics, indicating highly significant differences between the two groups.

Table 1 . Demographic and baseline physiological parameters.

Parameter	Healthy subjects	Stroke patients	p-values
Number (<i>n</i>)	237	98	-
Age (years)	51.3 $\pm$ 15.4	63.5 $\pm$ 12.7	<0.001
Sex, male (%)	123 (52%)	63 (64%)	0.044
CBFV (cm.s ⁻¹), R and AH	53.1 $\pm$ 14.5	45.8 $\pm$ 18.5	<0.001
CBFV (cm.s ⁻¹), L and UH	52.5 $\pm$ 13.1	45.9 $\pm$ 18.2	<0.001
MAP (mmHg)	87.7 $\pm$ 14.4	98.4 $\pm$ 14.3	<0.001
HR (bpm)	64.9 $\pm$ 10.6	71.1 $\pm$ 12.0	<0.001
EtCO ₂ (mmHg)	39.5 $\pm$ 7.0	33.9 $\pm$ 3.8	<0.001
Affected hemisphere (R/L, <i>n</i>)	-	49/49	-
NIHSS score	-	6.8 $\pm$ 5.9	-

Data are presented as *n* (% of available data), mean $\pm$ SD; R: right; L: left; AH: affected hemisphere; UH: unaffected hemisphere; CBFV: cerebral blood flow velocity; MAP: mean arterial blood pressure; HR: heart rate; EtCO₂: end-tidal CO₂; NIHSS: National Institute of Health Stroke Scale. p-values from independent t-tests.

Estimates of ARI were not accepted in 15 HS and 13 AIS, due to low values of the mean coherence in the 0.15-0.25 Hz frequency interval, or a NMSE>0.30, as described in Methods. As a consequence, all estimates of ARI included 222 HS (Table 2) and 85 AIS patients (Table 3).

Table 2 – Population TFA parameters in healthy subjects for different durations of signal segmentation.

Parameter		$S_D=100$ s	$S_D=50$ s	$S_D=25$ s	p-value ANOVA	ICC
Coherence (n=237)	VLF	0.45 ± 0.17	0.37 ± 0.18	0.37 ± 0.18	0.00001	0.852
	LF	0.64 ± 0.16	0.63 ± 0.16	0.57 ± 0.16	0.00001	0.870
Gain (%/%) (n=237)	VLF	1.04 ± 0.54	0.96 ± 0.54	0.95 ± 0.50	0.00001	0.934
	LF	1.57 ± 0.56	1.45 ± 0.54	1.32 ± 0.46	0.00001	0.914
Phase (rad) (n=237)	VLF	0.88 ± 0.44	0.86 ± 0.54	0.88 ± 0.50	0.60	0.783
	LF	0.64 ± 0.25	0.66 ± 0.26	0.69 ± 0.25	0.00001	0.850
ARI (n=222)		5.70 ± 1.61	5.70 ± 1.62	5.89 ± 1.52	0.0005	0.865

Values are mean $\pm$ SD. ARI: autoregulation index; VLF: very low frequency interval (0.02-0.07 Hz); LF: low frequency interval (0.07-0.20 Hz); p-value: one-way repeated measures ANOVA; ICC: intraclass correlation coefficient

Table 3 – Population TFA parameters in stroke patients for different durations of signal segmentation.

Parameter		$S_D=100$ s	$S_D=50$ s	$S_D=25$ s	p-value ANOVA	ICC
Coherence (n=98)	VLF	0.50 ± 0.19	0.47 ± 0.22	0.45 ± 0.22	0.00001	0.894
	LF	0.54 ± 0.22	0.51 ± 0.23	0.50 ± 0.21	0.00001	0.947
Gain (%/%) (n=98)	VLF	1.17 ± 0.74	1.14 ± 0.77	1.10 ± 0.72	0.01	0.957
	LF	1.39 ± 0.82	1.30 ± 0.74	1.24 ± 0.67	0.00001	0.930
Phase (rad) (n=98)	VLF	0.75 ± 0.64	0.71 ± 0.66	0.72 ± 0.63	0.18	0.950
	LF	0.66 ± 0.40	0.68 ± 0.40	0.64 ± 0.38	0.045	0.891
ARI (n=85)		5.1 ± 2.0	5.1 ± 2.1	5.1 ± 2.1	0.98	0.927

Values are mean $\pm$ SD. ARI: autoregulation index; VLF: very low frequency interval (0.02-0.07 Hz); LF: low frequency interval (0.07-0.20 Hz); p-value: one-way repeated measures ANOVA; ICC: intraclass correlation coefficient.

In HS, no significant differences between the right and left hemispheres were observed for CBFV, ARI, or the other spectral parameters and consequently mean values between the right and left sides

were adopted for all analyses. For AIS patients, only the affected side will be considered as discussed later.

3.1 Influence of signal segmentation on grouped data

In HS, the frequency response of coherence, gain and phase, as well as the CBFV response to a step change in BP, showed very similar curves for $S_D = 100, 50$, or 25 s, respectively (Fig. 2). With the exception of $\text{Phase}_{\text{VLF}}$, all other parameters in Table 2, showed significant differences for the three values of S_D considered (repeated measures ANOVA).

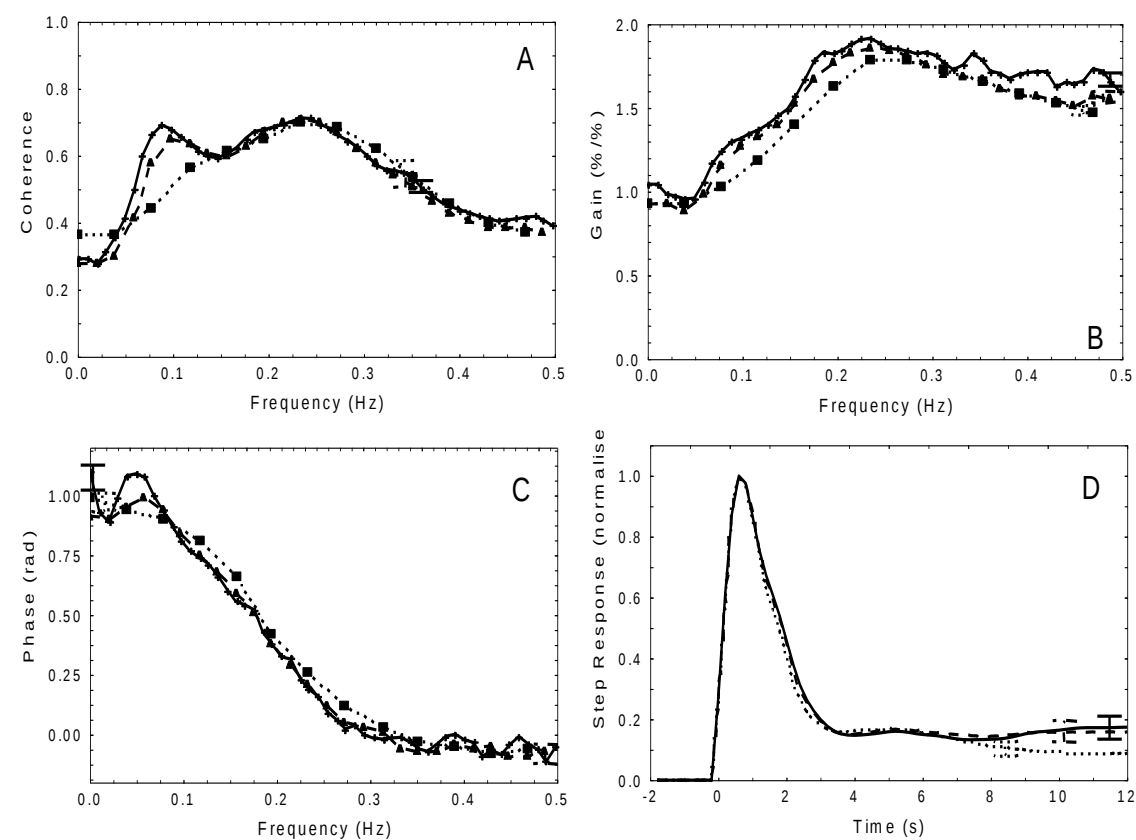


Figure 2 – Healthy subjects population average coherence (A), gain (B), phase (C) and CBFV response to a step change in BP (D) for segment durations of $S_D = 100$ s (crosses, continuous line), 50 s (triangles, dashed line) and 25 s (squares, dotted line), respectively. Error bars correspond to the largest ± 1 SE at the frequency or time of occurrence. Results shown for recordings from the right MCA. Nearly identical results were obtained for the left MCA.

Similar curves for AIS patients are given in Fig. 3, also showing excellent agreement for the three S_D conditions analysed. With the exception of $\text{Phase}_{\text{VLF}}$ and ARI, all other parameters showed highly significant differences for the effect of S_D .

As expected, the effects of S_D on frequency resolution ($\Delta f = 1/S_D$) are represented in Figs. 2 and 3 by the frequency interval between symbols, corresponding to $\Delta f \cong 0.01, 0.02$ and 0.04 Hz, for $S_D = 100, 50$ or 25 s, respectively.

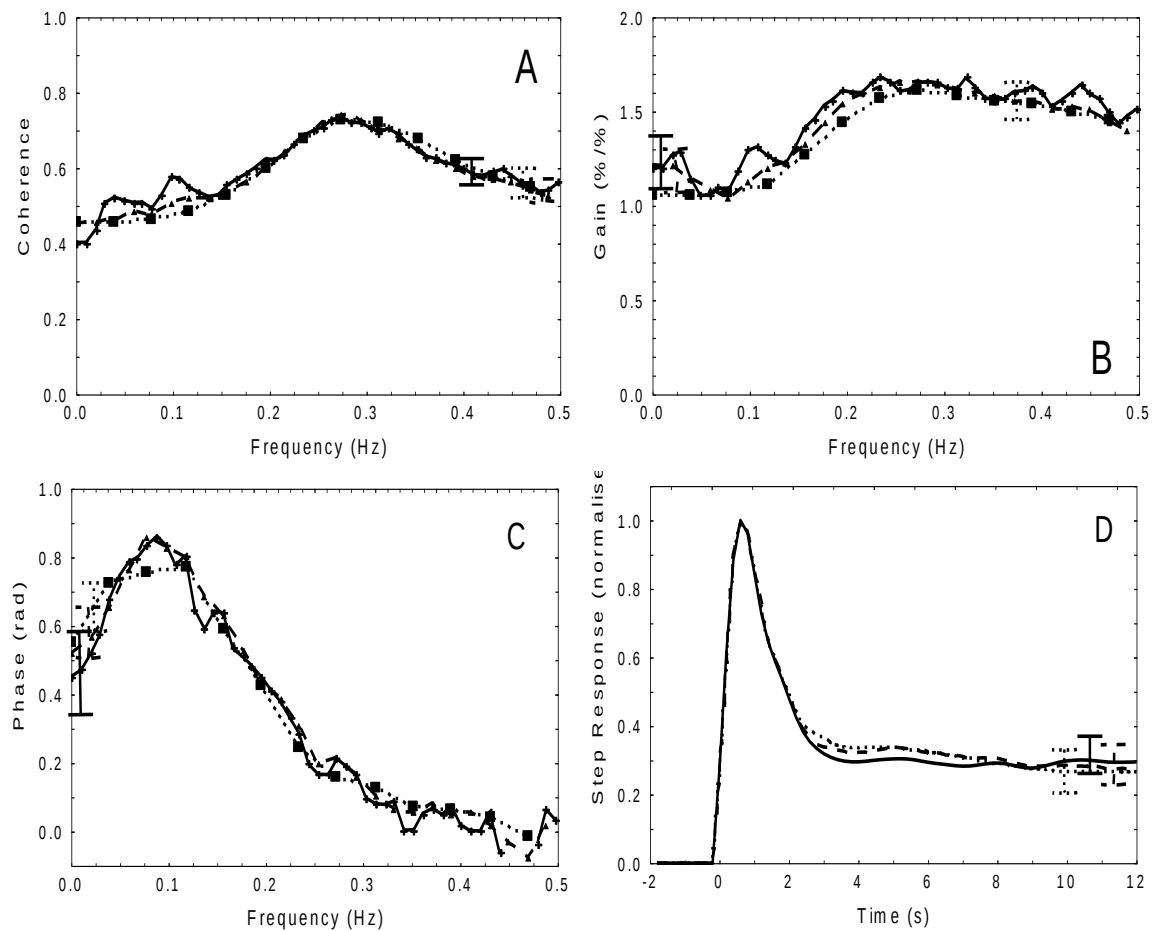


Figure 3 – Stroke patients population average coherence (A), gain (B), phase (C) and CBFV response to a step change in BP (D) for segment durations of $S_D = 100$ s (crosses, continuous line), 50 (triangles, dashed line) and 25 s (squares, dotted line), respectively. Error bars correspond to the largest ± 1 SE at the frequency or time of occurrence. Results shown for recordings from the affected hemisphere only.

For $S_D = 100$ s, ARI for AIS was significantly reduced compared to HS (Tables 2 & 3; $p=0.0073$). As indicated in Fig. 4, this difference between strokes and HS was maintained when S_D was set to either 50 ($p=0.0076$) or 25 s ($p=0.00042$), respectively.

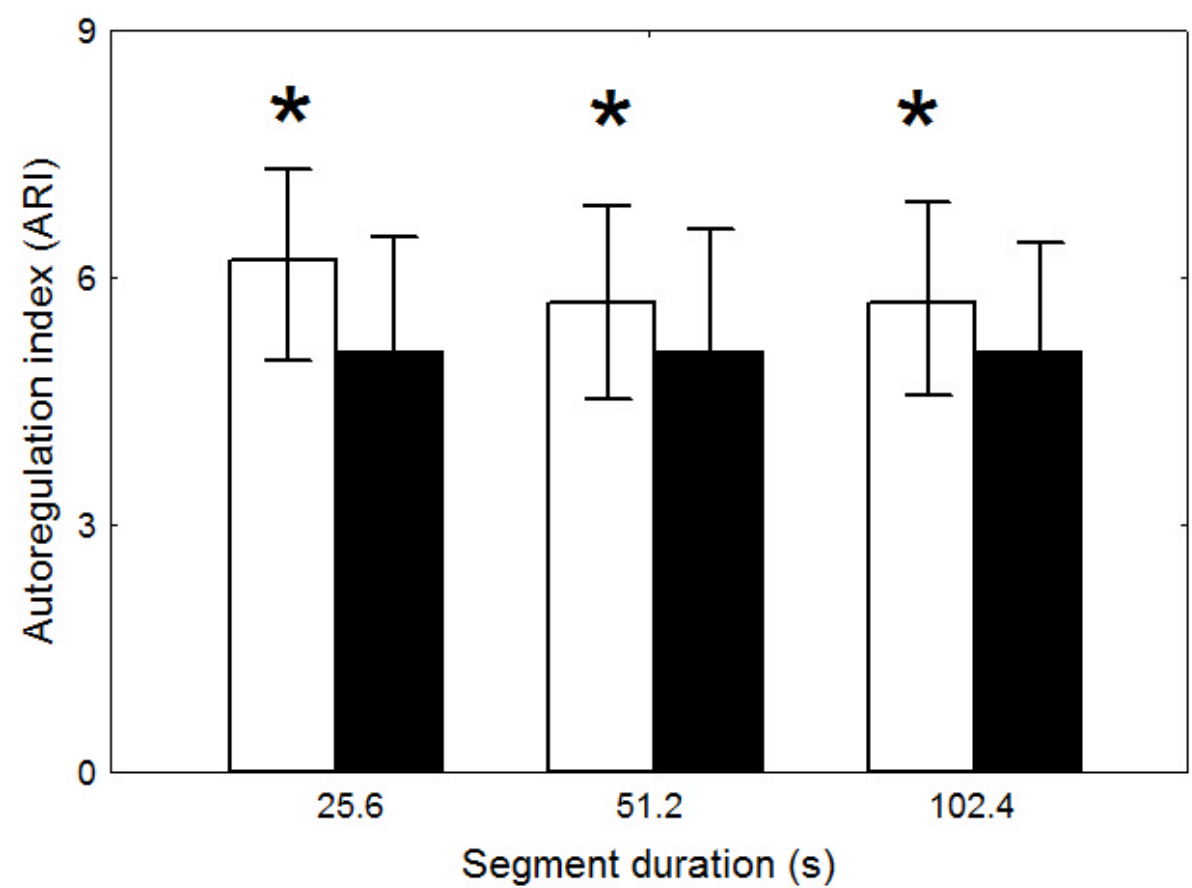


Figure 4 – Population median autoregulation index (ARI) of healthy subjects (light bars) and ischaemic stroke patients (dark bars) for the three segment durations considered, corresponding to 25 s, 50 s, and 100 s, respectively. Error bars represent the interquartile range. * $p<0.01$ for difference between healthy subjects and stroke patients within each segment duration.

For other TFA parameters, differences between AIS patients and HS, showed a mixed pattern. For each combination of S_D and frequency range (VLF or LF), coherence values were significantly different, but with coherence being lower in healthy participants in the VLF range and higher than patients in the LF range (Tables 2 & 3). $\text{Phase}_{\text{VLF}}$ was also significantly higher in HS compared to AIS patients, but in the LF range, phase did not show significant differences for any value of S_D . Gain_{VLF}

had significant differences only for $S_D = 50$ and 25 s, with AIS showing higher values than HC. This pattern was reversed for Gain_{LF} , which only showed a difference for $S_D = 50$ s.

3.2 Influence of signal segmentation on individual participants

The extent of consistency in intrasubject values of ARI, with different values of S_D , was assessed with the Bland-Altman plots in Fig. 5. For both HS and AIS patients, changes of S_D from 100 to 50 s (Figs. 5A&C) and from 50 to 25 s (Figs. 5B&D) had negligible biases and limits of agreement were around ± 1.5 units. For all parameters in Tables 2 and 3, ICC values were in the top range, corresponding to *excellent* reliability. Using ARI^{100} as reference, the rate of misclassification, based on a threshold of $\text{ARI} \leq 4$, was 6.3% for $S_D = 50$ s and 8.1% for $S_D = 25$ s in HS, with corresponding values of 11.7% and 8.2% in AIS patients, respectively.

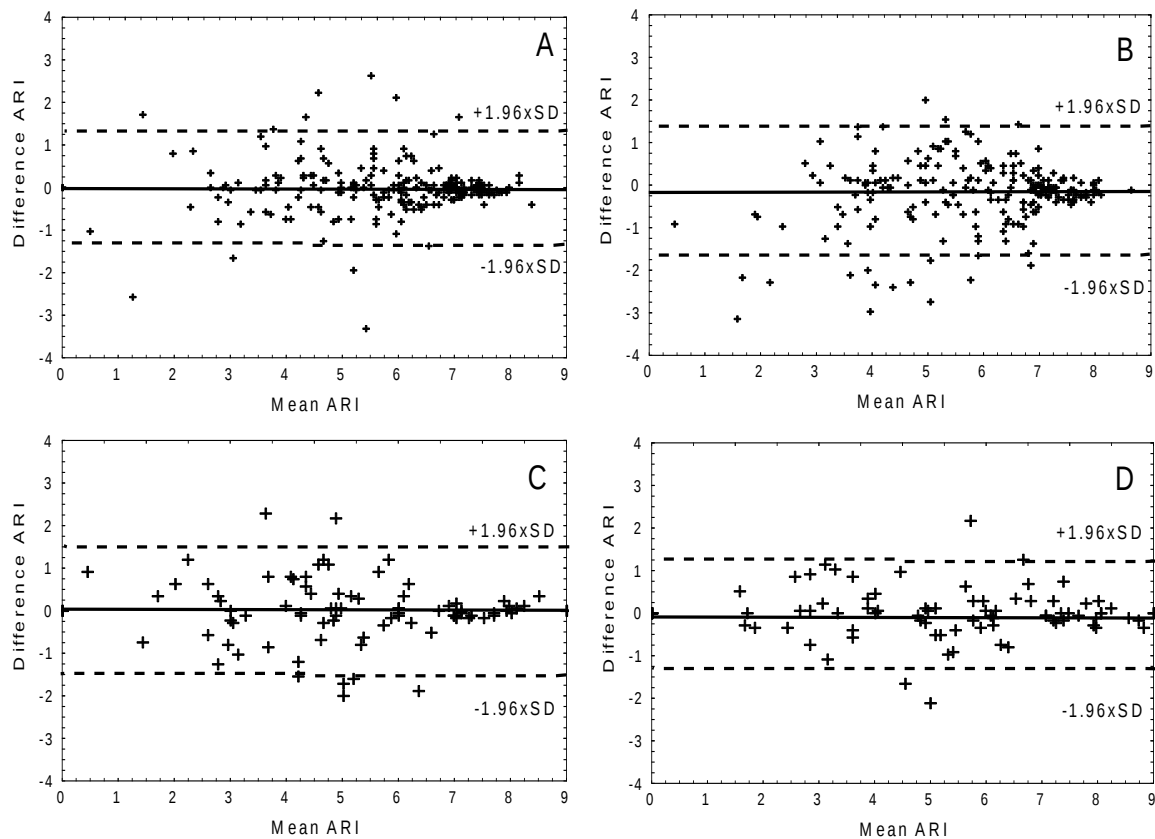


Figure 5 – Bland-Altman plots of agreement for the ARI index, comparing values obtained from healthy subjects (A,B) and the affected hemisphere from stroke patients (C,D). (A,C) ARI differences

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between estimates with $S_D = 100$ s and 50 s; (B,D) ARI differences between estimates with $S_D = 50$ s and 25 s, respectively. The bias is represented as a solid line and the limits of agreement as a dashed line.

3. Discussion

At first glance, the possibility of using different settings for S_D in TFA studies of dynamic CA, might look like a curiosity, or of little relevance, given the widespread use of 100 s for segmentation of CBFV and BP recordings in the literature (Claassen et al. 2016; Meel-van den Abeelen et al. 2014b). As discussed below though, the possibility of using different values of S_D could lead to further improvements in the sensitivity and reproducibility of metrics that are often used to express the efficiency of dCA using spontaneous fluctuations in BP.

3.1 Main findings

To our knowledge, systematic studies of the influence of S_D on parameters derived from TFA of BP and CBFV have not been reported previously. By providing detailed information on the influence of different settings of S_D on the main parameters derived by TFA, we aimed to address the lack of evidence in which further standardisation should be based (Claassen et al. 2016).

Strictly speaking, the main hypothesis formulated above should be rejected, as different values of S_D led to highly significant differences in most parameters (Tables 2 and 3), with the exception of the phase (VLF) in both groups and the ARI in AIS patients. Nevertheless, these exceptions, and a more detailed scrutiny of our results, can provide a different perspective on the use of different values of S_D . First of all, it is important to take into account the overall number of participants involved in both arms of the study, corresponding to a minimum of 222 HS and 85 AIS patients. Undoubtedly, the relatively high n provided enough statistical power to identify population differences that might not be of physiological or clinical relevance in all situations. As an example, despite the highly significant differences expressed by $p=0.0005$ in HS (Table 2), the mean value of ARI was 5.70 for both 100 and

50 s, increasing to 5.89 for $S_D = 25$ s. Similar considerations apply to the other parameters in Tables 2 and 3. Moreover, the excellent intrasubject reproducibility to changes in S_D , expressed by the ICC (Tables 2 & 3), would also suggest that different choices of S_D might be acceptable in both HS and AIS patients. On the other hand, intrasubject changes in ARI, resulting from different values of S_D , led to transitions across the $ARI \leq 4$ threshold adopted as a criterion of poor or impaired dCA. Other thresholds could be considered (Patel et al. 2016); the particular choice of $ARI \leq 4$ is representative of what might happen with different TFA settings. Although the rates of misclassification were relatively small, ranging from 6.3 to 11.7 %, a better understanding of their full implications will need much more extensive work, that might also involve assessment of the influence of other TFA parameters, such as total recording length, coupled with longer durations of segmentation, for example $S_D=200$ s.

Taken together, the different facets of our results suggest that values of S_D , different from the usual setting of 100 s, might be acceptable in particular circumstances, mainly when involving further methodological research into improving the reliability and prognostic value of dCA metrics in clinical applications.

3.2 Methodological considerations

A previous multi-centre study examined the influence of S_D on a single ‘recording’, comprising surrogate data corresponding to $ARI=6$ (Meel-van den Abeelen et al. 2014a). Although limited in scope, their results were in broad agreement with ours, for a range of values of S_D from 95 to 25 s.

Assessment of dCA based on spontaneous fluctuations of BP and CBFV is the ideal approach for physiological and clinical studies, given its general acceptability, including critically ill patients, and minimal interference with underlying physiological processes. However, the poor reproducibility of this method has been of considerable concern (Birch et al. 2002; Brodie et al. 2009; Sanders et al. 2018; Simpson and Claassen 2018; Tzeng and Panerai 2018). More recently, the causes for this unsatisfactory reproducibility have been ascribed more to the underlying variability of the different physiological mechanisms involved, rather than to the different methods that can be used to quantify dCA (Panerai 2013; Sanders et al. 2019). Before this view becomes enshrined though, there are

further methodological aspects that should be pursued and these include the investigation of dCA reproducibility with different values of S_D . The main explanations for reduced values of S_D leading to improvements in dCA reproducibility are twofold. Firstly, with values of $S_D = 50$ s or 25 s, the extent of spectral smoothing that can be obtained in estimates of gain, phase and ARI increases considerably, from using only $N_{SEG} = 5$ with $S_D = 100$ s, to as many as $N_{SEG} = 23$ with $S_D = 25$ s. Secondly, with a much larger number of data segments available, it might be possible to improve reproducibility by removing short segments of data, e.g. 25 s, that might either include artefacts, or represent periods of BP-CBFV ‘uncoupling’ due to the interference of co-variates, such as alterations in breathing, alert reactions or underlying neural activation (Panerai 2013). This line of investigation is underway in our group and we hope to report its outcome in the near future.

One important methodological consideration, when using the 95% confidence limits for coherence as a criterion for acceptance of ARI estimates (Fig. 1), is the choice of the 0.15-0.25 Hz frequency region for calculation of the confidence limits. In the VLF and LF regions, dCA is active and therefore involves a highly non-linear relationship between BP and CBF(V) due to changes in cerebrovascular resistance resulting from vasomotion (Panerai et al. 2006). The choice of the 0.15-0.25 Hz frequency band represents a compromise between a region where dCA is no longer active, and hence linear, and the tail of the BP spectral power distribution where there is enough signal-to-noise ratio to allow reliable estimates of coherence (Panerai et al. 2016).

3.3 Clinical implications

Studies of dCA in stroke have consistently reported significant differences in TFA parameters, when compared to healthy controls, although the strength of association can be influenced by stroke location, severity and stage of recovery (Aries et al. 2010; Intharakham et al. 2019a; Llwyd et al. 2018; Ma et al. 2016; Panerai 2008; Salinet et al. 2014; Salinet et al. 2019b). The inclusion of a relatively large number of AIS patients in this study did not aim to revisit the findings of those former investigations, but only to report on the influence of different values of S_D on a dataset representative of the different conditions and challenges encountered when making physiological measurements in a

clinical setting, where individuals might be sedated, agitated or not able to cooperate. Overall, our findings were not strikingly different from those observed in HS. Of note though, in both groups of individuals, the $\text{Phase}_{\text{VLF}}$ and, in AIS patients, the ARI index, were not influenced by the choice of S_D (Table 3). This is remarkable because, coincidentally, the phase and the ARI index are the two metrics that have showed greater consistency in detecting differences in TFA parameters in stroke, when compared to healthy controls (Intharakham et al. 2019a; Panerai 2008). Moreover, the significant differences observed between HS and AIS patients were not affected by changes in S_D (Fig. 4). In the particular case of our study, mean values of ARI for AIS were approximately 5 (Table 3) which, on average, would be regarded as ‘normal’ according to the original scale proposed by Tiecks et al (Tiecks et al. 1995). However, the Tiecks et al scale was based on ARI estimates derived from thigh cuff manoeuvres (Tiecks et al. 1995), whilst the values we normally obtain from the CBFV step response estimated by TFA, tend to be around 6 (Patel et al. 2016). In our case, the relatively small difference in ARI values between the healthy group and AIS patients were likely affected by two other factors. Firstly, the AIS population was dominated by mild strokes, as reflected by a mean NIHSS = 6.8 and, secondly, as observed in many other studies, AIS patients were hypocapnic (Table 1), which was likely to have improved their autoregulatory efficiency (Minhas et al. 2018; Salinet et al. 2019a).

Although recordings with a minimum duration of five minutes should be obtained whenever possible (Claassen et al. 2016), in clinical settings this might be problematic, mainly in critically ill patients. As recently reported though (Intharakham et al. 2019b), in exceptional circumstances, shorter measurement durations, as low as three minutes, might be acceptable. In this context, the use of reduced values of S_D will be not only necessary, but also desirable to provide sufficient smoothing, comparable to what is usually obtained with five-minute recordings.

Based on the findings of this study, and the considerations above, it would be pertinent to suggest that further work should investigate whether different values of S_D could lead to greater sensitivity and prognostic value of the ARI, and other TFA parameters, to detect deterioration of dCA in stroke and other cerebrovascular conditions.

3.4 Limitations

Studies based on TCD measurements are usually limited by the assumption that the cross-sectional area of the insonated vessel should remain constant, to provide a strong association between CBFV and CBF. In our particular case though, this concern is less relevant since the hypothesis tested did not involve longitudinal comparisons and values of EtCO₂ did not reach the extremes where changes in MCA diameter could be expected (Coverdale et al. 2014; Verbree et al. 2014).

As stated above, the main objective of studying two different groups of individuals was to assess the influence of S_D within each group. In the case of AIS patients though, it was also relevant to report how differences in dCA parameters, in comparison with HS, behaved with different values of S_D. This aspect of the study could be limited by differences in demographic and baseline physiological parameters between the two groups (Table 1). As mentioned above, the reduced EtCO₂ in AIS would suggest that the dCA in these patients was improved compared to that expected during normocapnia. HS were younger, but age has not been shown to affect dCA performance (van Beek et al. 2008). Hypertension is a well-known risk factor for stroke and is prevalent in most retrospective studies. Reduced CBFV has been consistently reported in AIS when compared to controls, including prospective studies matched for age and BP (Gommer et al. 2008; Saeed et al. 2013). On the other hand, the potential contribution of HR, as a co-factor for dCA alterations in AIS, have not been reported and deserve further investigation, mainly as a proxy for increased sympathetic activity.

In the AIS group, we studied only the affected hemisphere for two main reasons. Firstly, this would be the side where we could expect a greater challenge in recording good quality signals of CBFV, due to alterations in perfusion. Secondly, because the unaffected hemisphere would be unlikely to contribute with relevant additional information, either with an equally altered dCA, as reported in many previous studies (Xiong et al. 2016), or in the case of dCA being normal, thus behaving similarly to the HS group (Salinet et al. 2019b).

The strict criteria adopted for acceptance of estimates of ARI (Panerai et al. 2016), led to the rejection of 15 HS and 13 AIS patients, corresponding to 6.3% and 13.2% of the total number of participants in

each group, respectively. Noteworthy, although non-significant, a smaller number of participants was removed for $S_D=25$ s, for both HS and AIS, but to keep the datasets comparable for different values of S_D , once a recording was removed for one setting, it was also removed from all three settings of S_D .

When obtaining estimates of coherence, gain and phase in the VLF region, covering the 0.02-0.07 Hz frequency range, it is important to note that the lowest harmonic available for $S_D = 25$ s is 0.04 Hz, with the implication that the 0.02 Hz and 0.03 Hz harmonics are thus not included. In principle this limitation would also affect estimates of ARI, due to the difficulty of including the contribution of very low frequency fluctuations in BP and CBFV. Although a reduced contribution of VLF in estimates of ARI could explain the higher values obtained for $SD = 25$ s in HC (Table 2), this was not reflected in either individual (ICC values) or values of ARI in AIS (Table 3).

4. Conclusions

Different durations of data segments used in TFA studies of dCA, based on Welch's method, can lead to different values of the ARI, coherence, gain and phase in healthy subjects, but in AIS patients, the ARI and phase were not altered. When taking into account the physiological and clinical relevance of the differences observed, as well as the excellent intraclass correlation coefficient for the different durations considered, it can be concluded that segment durations, shorter than the recommended standard of 100 s, should be a valid alternative in future investigations aiming to improve the reproducibility, sensitivity or prognostic value of dCA metrics derived by TFA of spontaneous fluctuations in BP and CBFV.

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