IN CONTROL

Methodological and clinical aspects of cerebral autoregulation and haemodynamics.

Aisha Meel-
van den Abeelen

DONDERS
series

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In control

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CONTENTS

ABBREVIATIONS

AD	Alzheimer's disease
ARXAR model	bivariate causal model
AUC	area under the curve
BMI	body mass index
BP	blood pressure
BPV	blood pressure variability
BR	baroreflex
BRS	baroreflex sensitivity
CA	cerebral autoregulation
CBF	cerebral blood flow
CBFV	cerebral blood flow velocity
CCM	convergent cross mapping
ChEI	cholinesterase inhibitors
CIRS-G	cumulative illness rating scale for geriatrics
CoV	coefficients of variation
CRP	c-reactive protein
CSH	carotid sinus hypersensitivity
CSM	carotid sinus massage
CVCI	cerebrovascular conductance index
CVCI	cerebrovascular conductance index
CVD	cardiovascular disease
CVMR	cerebral vasomotor reactivity
DPF	differential pathlength factor
ECG	electrocardiogram
FFT	fast Fourier transform
fNIRs	functional near-infrared spectroscopy
HbDiff	oxygenation index
HF	high frequency
HFO	high frequency oscillations
[HHb]	deoxygenated haemoglobin concentration
HR	heart rate
HRV	heart rate variability
IL-6	interleukin -6
LF	low frequency

LFOs	low frequency oscillations
LPS	*E. coli lipopolysaccharide*
MCA	middle cerebral artery
MCI	mild cognitive impairment
MUMC	Maastricht University Medical Center
NIRS	near-infrared spectroscopy
NR	not reported
[O2Hb]	oxygenated haemoglobin concentration
OH	orthostatic hypotension
PPH	postprandial hypotension
RMSSD-HRV	square root of the mean-squared difference of successive heart beat intervals
ROC	receiver operating characteristic
ROC	receiver operating characteristic
RRi	R-R interval; time between two successive heart beats
RUNMC	Radboud University Nijmegen Medical Centre
SBP	systolic blood pressure
SDNN-HRV	standard deviation of all normal heart beat intervals
TCD	transcranial Doppler
TFA	transfer function analysis
[tHb]	total haemoglobin concentration
TNF-α	tumor necrosis factor alpha
VLF	very low frequency
VLFOs	very low frequency oscillations

Chapter 1.

General introduction and outline

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BACKGROUND

Cerebral perfusion

The human brain is a complex organ that is critically dependent on its blood supply. It comprises only about 2 % of the total body weight. However, it consumes around 20 % of the total available oxygen for normal functioning [1]. This makes the brain one of the most highly perfused organs in the body. Unlike the kidney, liver or muscle, the brain is only able to withstand very short periods of inadequate oxygen supply. Insufficient blood flow and oxygen will result in cerebral ischemia, in which the neurons and other brain cells are damaged and lose their function. On the other hand, excessive blood perfusion may also have unfavourable consequences, such as intracranial hypertension or capillary damage. Maintenance of an adequate cerebral perfusion is therefore critical to ensure a sufficient delivery of oxygen and glucose and to avoid brain injury. As a result, regulatory mechanisms act to control systemic blood pressure and cerebral blood flow. This way, even under considerable external changes, an adequate blood- and oxygen supply to the brain is maintained in accordance with its underlying functional and metabolic needs. The most important aspects of the body's perfusion regulation consist of the integrated control of systemic blood pressure and cerebral blood flow via the arterial baroreflex and cerebral autoregulation, respectively [3, 4]. However, literature shows that different pathological conditions, such as dementia, stroke and head trauma [5-9], may influence these highly important regulation systems. In subjects with a disturbed brain perfusion regulation, the brain may be excessively sensitive to fluctuations in blood pressure, which has been associated with increased morbidity and mortality [10, 11].

Maintaining safe levels of cerebral perfusion is thus essential to preserve cerebral function. Therefore, the ability to accurately quantify the quality of the perfusion regulation is of importance in clinical practice. Monitoring the quality of brain perfusion may be of benefit in the care of patients with brain injury, meningitis or stroke. But it may also be of importance for early detection of, for example, neurodegenerative diseases.

The two mechanisms that act together to safeguard brain perfusion, blood pressure control (the baroreflex mechanism) and cerebral autoregulation, will be discussed below.

Blood pressure: the baroreflex

The baroreflex is a reflex loop with cardiac, vascular and cerebral components involved in short-term blood pressure regulation [12]. The baroreflex works through the baroreceptors, which are stretch-sensitive fibres embedded primarily in the wall of the carotid arteries and aorta. Changes in blood pressure lead to changes in the arterial vessel wall, which are sensed by the baroreceptors and information is sent to the brainstem. Via the autonomic nervous system heart rate and vascular tone are changed to restore the blood pressure. A clinical example is the drop in blood pressure upon standing (Figure 1), which the baroreflex corrects by a rapid increase in heart rate (parasympathetic inhibition) followed by peripheral arterial vasoconstriction (sympathetic activation). Abnormalities in the vascular baroreceptors, the glosso-pharyngeal or vagal nerves, or the brain stem could lead to impairment of the baroreflex. Baroreflex failure may result in a significant dysregulation of blood pressure, leading to increased blood pressure variability. This may result in sudden pressure drops on shifting from supine to standing position as well as aberrant pressure rises with a major risk of fatal events such as myocardial infarction and stroke. The quality of the baroreflex function can, for example, be assessed by evaluating the relationship between variations in heart rate and blood pressure [13-15].

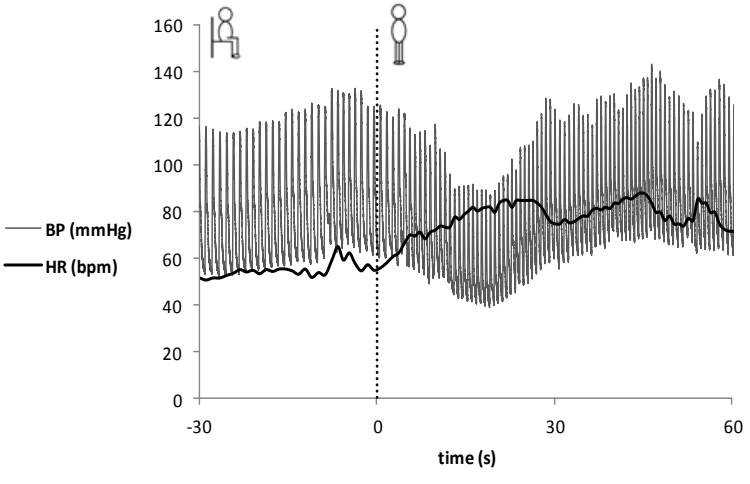

Figure 1. Example of blood pressure (grey area, the upper and lower borders corresponding to the systolic and diastolic blood pressures) and beat-to-beat heart rate (thin black line) of a healthy person before and after standing up. BP= blood pressure. HR = heart rate.

Cerebral autoregulation

Cerebral autoregulation acts to maintain a relatively constant cerebral blood flow despite fluctuations in blood pressure. Cerebral autoregulation is achieved by changes in cerebral vascular tone in response to changes in intravascular pressure: when the blood pressure decreases the radius of the cerebral vessels increases (vasodilation; increasing the cerebral blood flow) and when the blood pressure increases the radius decreases (vasoconstriction; decreasing the cerebral blood flow). This autoregulatory mechanism was first proposed by Lassen et al. [4]. They proposed that cerebral autoregulation works within a certain range of blood pressures (≈ 60 to 150 mmHg). Outside this so-called autoregulatory range, vasomotor adjustments are exhausted and cerebral blood flow becomes pressure-passive and subjected to changes in blood pressure (Figure 2). Nowadays, this view on cerebral autoregulation is called 'static autoregulation' and is often studied using interventions inducing (large) blood pressure fluctuations, for example, by administering drugs that increase (phenylephrine) or decrease (sodium nitroprusside) blood pressure.

Over the last two decades, techniques with a high temporal resolution (> 10 Hz) have been developed which allow analysis of the amplitude and time latencies of the cerebral blood

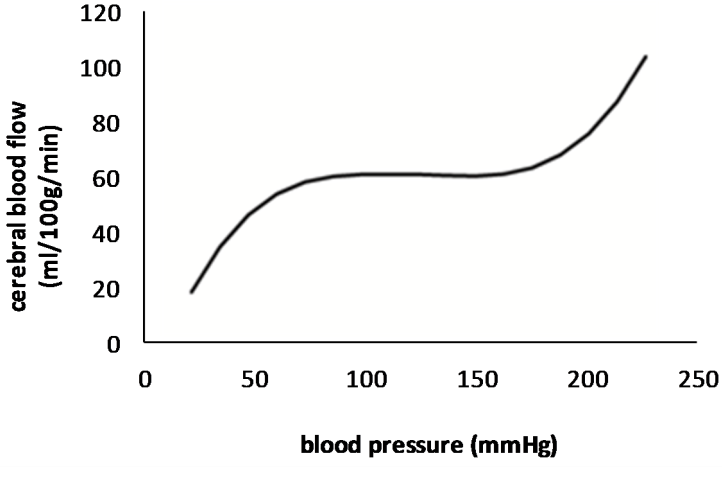

Figure 2. Static autoregulation curve.

During intact cerebral autoregulation, cerebral blood flow becomes only pressure passive when blood pressure comes below the lower limit or above the upper limit (based on [1]).

flow response to rapid (seconds) changes in blood pressure. One of these techniques is transcranial Doppler sonography, which uses a piezoelectric crystal probe placed on the temporal window of the skull. Ultrasound waves are sent through the skull and reflections of these waves on flowing blood result in a frequency shift of the sound. This frequency shift is used to quantify the cerebral blood flow velocity, which can be used as a surrogate for the cerebral blood flow.

With high temporal resolution (> 10 Hz) techniques, such as transcranial Doppler, it was shown that sudden changes (elevation and reductions) in blood pressure are transmitted directly to the brain circulation under usual circumstances, but within a brief amount of time brain blood flow tends to return to its baseline value. This observation suggests that the relationship between cerebral blood flow and blood pressure within the autoregulatory range is not completely flat. The fast mechanisms that permit the restoration of cerebral blood flow after a perturbation in blood pressure are referred to as 'dynamic cerebral autoregulation' [16]. In subjects with a disturbed dynamic cerebral autoregulatory functioning, the brain may be excessively sensitive to short-term fluctuations in blood pressures.

Several methods of analysis, involving a diversity of protocols, measurement techniques and data analysis approaches, have been developed for non-invasive assessment of dynamic cerebral autoregulation. These techniques can be split up into time domain (i.e. correlation index [17]), frequency domain (i.e. transfer function analysis [18]) and non-linear measures (i.e. Laguerre expansions of Volterra kernels [19]).

AIM OF THIS THESIS

Accumulating evidence indicates that, in clinical situations, information from baroreflex functioning and dynamic cerebral autoregulation is crucial for correct interpretation of the impact of severe interventions or events that may threaten the vulnerable brain tissue [5, 20-22]. Keeping the blood pressure at an adequately stable level, by careful monitoring and rapid correction, may be of great importance in such circumstances.

Despite the importance of measuring the cerebral autoregulatory performance, currently no gold standard test of autoregulation exists that may be performed safely and easily in a wide sphere of clinical conditions. Many uncertainties exist with regard to the applied methods, making it difficult to replicate or compare the results of different studies, and this further hinders the applicability in clinical practice.

In the first part of this thesis, we aim to obtain better insight into the quantification of cerebral autoregulation. A special focus is placed on the most often applied non-invasive technique for the analysis of cerebral autoregulation, namely transfer function analysis. The first main research question is:

How is transfer function analysis applied
for the quantification of cerebral autoregulation?

By performing an extensive literature search and through debate with experts in the field, the current state of the art was reviewed. All this was done with the ultimate goal to come to a consensus agreement on how to quantify cerebral autoregulation.

In the second part, the emphasis is shifted to haemodynamics in clinical practice. We aim to investigate whether perfusion regulation is changed in different pathophysiological conditions. Inspired by the clinical background of the department where this thesis research was performed, we chose conditions that are of relevance for an elderly population. The second main research question is :

Is the perfusion regulation impaired
in patients with Alzheimer's disease, frail elderly and/or during systemic inflammation?

OUTLINE OF THIS THESIS

The two general aims have been translated in a series of background studies, clinical experiments and retrospective analysis which are presented in the subsequent chapters of this thesis.

Part 1. Quantification of cerebral autoregulation (Chapter 2 to 5)

Research question:
How is transfer function analysis applied for the quantification of cerebral autoregulation?

Chapter 2 provides a systematic review on transfer function analysis, the most widely used method for the quantification of cerebral autoregulation based on spontaneous oscillations in blood pressure and cerebral blood flow velocity. The mathematical background of the method is described and an overview is given of how the method is applied by different researchers. One hundred thirteen articles were included and, specifically, the enormous heterogeneity in outcome values was addressed.

Chapter 3 dives deeper into the variations found in the application of the transfer function analysis for quantification of cerebral autoregulation. A multi-centre study was performed to provide insight into the between-centre variation in transfer function outcomes. Next to the examination of clinical data, artificial datasets were used to examine the effect of different parameter settings on transfer function outcomes.

Chapter 4 proposes a consensus for international guidelines on transfer function analysis for the quantification of cerebral autoregulation.

Chapter 5 introduces a new non-linear method for the quantification of cerebral autoregulation. Despite the fact that transfer function analysis is the most used method in literature for the quantification of cerebral autoregulation, transfer function analysis may not be able to cover the whole process of cerebral autoregulation as it is based on the assumption that cerebral autoregulation is a linear process. However, it has been observed that the coherence function between blood pressure and cerebral blood flow is reduced below 0.07 Hz [23], indicating intrinsic non-linearities and/or non-stationarities in this frequency range [24]. This observation and the fact that the static autoregulation curve is non-linear make that one cannot confidently ascertain that cerebral autoregulation is not a

non-linear process. The usage of non-linear methods may thus provide a broader notion of the mechanism pertinent to cerebral autoregulation [25]. This study investigates the usage of the non-linear analysis technique, convergent cross mapping, for the quantification of cerebral autoregulation.

Part 2. Clinical application of haemodynamic analysis (Chapters 6 to 11)

Part 2.1. Alzheimer's disease

Research question:
Is the perfusion regulation impaired in patients with Alzheimer's disease?

Alzheimer's disease, the leading cause of dementia, is a progressive neurodegenerative disorder. There is still a limited understanding of this disease and its underlying cause. A growing body of evidence points towards vascular pathology involvement in the disease. This vascular hypothesis states that systemic and cerebral vascular effects contribute to neurodegeneration and development of Alzheimer's disease.

Chapter 6 investigates the cerebral autoregulation in patients with Alzheimer's disease. In addition to the transfer function analysis, cerebral autoregulation was assessed by investigating the effect of (repeated) sit-stand manoeuvres on blood pressure and cerebral blood flow velocity. Next to the cerebral autoregulation, the cerebral vasomotor reactivity was investigated, which is a mechanism that reflects the uniquely strong response of cerebral blood vessels to changes in arterial carbon dioxide concentration.

Chapter 7 continues the research in the vascular hypothesis for Alzheimer's disease. This study explores the role of the baroreflex functioning in the pathophysiology of Alzheimer's disease.

Part 2.2. Frail elderly

Research question:
Is the perfusion regulation impaired in frail elderly?

Aging is associated with physiological changes of the vascular system. The systemic and haemodynamic regulation systems may be affected, creating a higher risk of cerebral

hypo- and hyperperfusion.

Chapter 8 examines the effect of aging itself on cerebral haemodynamics.

Hypertension affects 20% to 30% of the world population and is the most prevalent modifiable risk factor for stroke. Long-standing hypertension may result in structural changes of the cerebral vessels, such as thickening of the vessel walls with narrowing of the lumen and hyalinosis of the media resulting in stiffness.

Chapter 9 describes how hypertension in elderly influences the baroreflex function, cerebral autoregulation and cerebral vasomotor reactivity.

Also among elderly, the prevalence of orthostatic hypotension, postprandial hypotension and carotid sinus hypersensitivity is high. These disorders of blood pressure regulation may cause severe cerebral hypoperfusion, causing symptoms as weakness, dizziness and syncope. Orthostatic hypotension is predominantly seen as a disorder of autonomic failure and postprandial hypotension and carotid sinus hypersensitivity are classified as reflex or neurally mediated syncope. As the cardiovascular autonomic system plays an important role in the distribution of blood volume and the regulation of blood pressure, failure of this system might play an important role in the aetiology and pathophysiology of these hypotensive syndromes.

Chapter 10 investigates whether orthostatic hypotension, postprandial hypotension and/or carotid sinus hypersensitivity are related to changes in heart rate variability, blood pressure variability and/or baroreflex functioning.

Part 2.3. Inflammation

Research question:
Is the perfusion regulation impaired during systemic inflammation?

Sepsis is a systemic host response to a severe bacterial infection, characterized by a widespread state of inflammation, often complicated by organ dysfunction or failure. It is a potentially deadly medical condition, often accompanied by irreversible acute cerebral dysfunction. Despite the fact that the exact pathophysiology remains unknown, many indicators, such as reduced global perfusion, disruption of the blood-brain barrier and

cerebral edema, point towards a link between cerebral perfusion and brain dysfunction.

Chapter 11 describes the use of purified *E. coli* lipopolysaccharide, as an established human in vivo model of the systemic inflammatory response that occurs during early sepsis, to assess the effect of the systemic inflammatory response on cerebral autoregulation functioning.

General discussion and summary

Chapter 12 provides a general discussion of the findings in this thesis.

Chapter 13 provides a summary of the chapters in this thesis.

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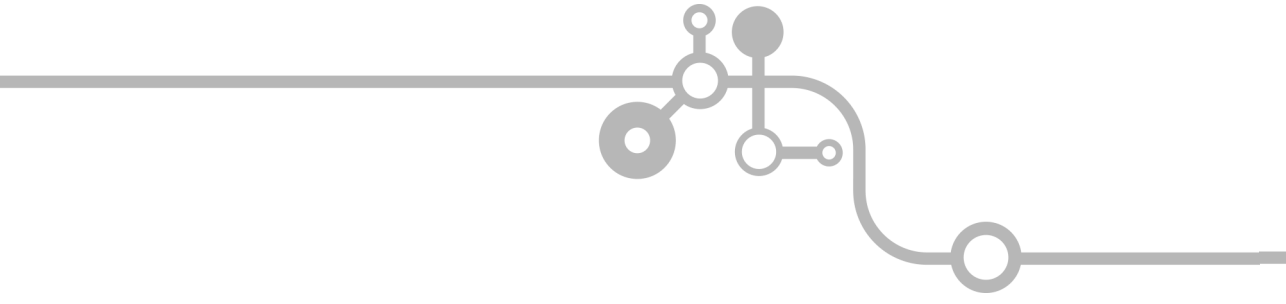

Part 1.

Quantification of cerebral autoregulation

Chapter 2.

Transfer function analysis for the assessment of cerebral autoregulation

Medical Engineering & Physics. 2014 May ; 36(5):563-575

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ABSTRACT

Cerebral autoregulation (CA) is a key mechanism to protect the brain against excessive fluctuations in blood pressure (BP) and maintain cerebral blood flow. Analysing the relationship between spontaneous BP and cerebral blood flow velocity (CBFV) using transfer function analysis is a widely used technique to quantify CA in a non-invasive way. The objective of this review was to provide an overview of transfer function techniques used in the assessment of CA.

For this review, 113 publications were included. This literature showed that there is no gold standard for the execution and implementation of the transfer function. There is a high diversity in settings and criteria used for transfer function analysis. Notable is also the high number of studies which report little on the settings.

This disparity makes it difficult to replicate or compare the results of the different studies and further hinders the opportunity to make a distinction between intact and impaired CA in different patient groups.

More research on the effects of different implementation techniques on outcomes for CA and optimization of the transfer function analysis is urgently needed. Furthermore, the results of this review show that international guidelines should be created to inform the minimal description of the applied technique and the interpretation of transfer function outcomes in scientific research.

INTRODUCTION

Cerebral autoregulation

Cerebral autoregulation (CA), first introduced by Lassen et al. [8], refers to the intrinsic ability of the brain to stabilize cerebral blood flow despite changes in blood pressure (BP) [10, 11]. CA is a key protective mechanism of the brain, and has an important role during both physiological and pathological situations [12]. A reduction in CA has been reported in for example carotid artery disease [13], severe head injury [14], ischemic stroke [15], hypertension [16], Parkinson's disease [19] and obstructive sleep apnoea [20]. In subjects with a disturbed CA, the brain may be excessively sensitive to fluctuations in BP. Autoregulation failure has been associated with increased morbidity and mortality [21]. However, the underlying mechanisms that cause the impairments in CA are not yet fully understood.

Dynamic cerebral autoregulation

For a long time, CA was considered as a static phenomenon [23], namely the regulation of cerebral blood flow during gradual changes (minutes – days) in BP. Evaluation of CA was performed by investigating the difference in cerebral blood flow before and after the autoregulatory response to a manipulation in BP. If the cerebral blood flow changed significantly, CA was said to be impaired. If cerebral blood flow remained nearly constant, CA was said to be intact [25]. Over the last two decades, the high temporal resolution of transcranial Doppler (TCD) sonography allowed analysis of the amplitude and time latencies of the cerebral blood flow velocity (CBFV) response to rapid (seconds) changes in BP [26]. It was shown that, under normal conditions, CBFV tends to return to its original value with a time constant of a few seconds. The evidence that cerebral blood flow, after a perturbation, requires a finite amount of time to return to its original value has led to the distinction between 'static' and 'dynamic' CA [28]. This dynamic approach quantifies the fast modifications in cerebral blood flow in relation to rapid alterations in BP within the upper and lower limits of static CA and reflects the latency and efficiency of the cerebral vasoregulatory system [35]. In contrast to static CA, dynamic CA allows differentiation of the CA responses to fluctuations in BP of different amplitudes and durations, representing daily life challenges for CA [25]. Non-invasive evaluation of CA could be a source of valuable information for clinical management.

Methods to measure dynamic cerebral autoregulation

Induced changes / challenges

34 In order to induce changes in BP and CBFV, and thus to quantify dynamic CA, several methods have been developed. The traditional techniques assess CA by challenging the cerebrovascular systems using interventions such as the cold pressor test, squat-to-stand and/or sit-to-stand manoeuvres and the deflation of thigh cuffs [35-37]. These interventions induce (large) BP fluctuations; however, they require cooperation of patients and can be uncomfortable, making them unsuitable in cases of severe illness or in older or cognitively impaired persons. Furthermore, the interventions might affect other physiological subsystems (e.g. sympathetic activation with the cold pressor test) or parameters (e.g. pCO_2 with squat-to-stand and/or sit-to-stand [35]), confounding the results. This limits their value for daily practice in a clinical setting in a broad range of patients.

Spontaneous changes

Fortunately, several methods of analysis, involving a diversity of protocols, measurement techniques, and data analysis approaches, have been developed for non-invasive assessment of dynamic CA in the resting state [12]. These techniques use spontaneous fluctuations in BP and CBFV. The advantages of using spontaneous oscillations are that they do not need additional clinical manoeuvres, are less laborious and may be used in a wider range of patients, including those who are unstable or unable to cooperate with or tolerate the challenges required to provoke a haemodynamic response [12]. Moreover, they allow continuous, non-invasive monitoring for cerebrovascular function. Of all available methods to do this, the transfer function analysis is the most frequent method reported in literature to quantify CA using spontaneous fluctuations. The transfer function method, first carried out to quantify dynamic CA by Giller et al. [39], analyses the relationship between the oscillations in BP and CBFV in the frequency domain.

Aim of this study

The first objective of our investigation is to provide an overview of the variations in the transfer function technique as it is used in the scientific literature to assess dynamic CA for spontaneous oscillations in BP and CBFV. The hypothesis was that there were variations in transfer function methods between countries and between centres, and that these variations make direct comparisons between studies difficult if not impossible. The second objective was to assess the reproducibility and potential utility as a clinical test of the transfer function

analysis for CA as reported by multiple investigators. Since previous studies on CA have used relatively small sample sizes, the aim was to combine results of multiple studies to investigate the inter-study variability in CA values for healthy subjects and to obtain insight into the discriminative power of the transfer function analysis between physiological and pathological conditions.

METHODS

Literature search

To retrieve the studies that had used transfer function analysis to assess dynamic CA for spontaneous BP and CBFV oscillations, an online search of the literature (PubMed, Embase and WebOfScience) was conducted on October the 15th 2012, including only articles written in English. Search terms included are shown in Table 1. Two authors (AM, AB) independently assessed eligibility by reading abstracts and, if necessary, whole articles. The snowball method was used to manually identify relevant references from the reference lists of included articles. Animal studies or articles that used any manoeuvre or intervention to change BP and quantify CA were excluded from the search. The following data were extracted from the remaining articles: number of included subjects, health status, physiological condition used, status of CA and technical aspects of the transfer function analysis used to assess CA.

Table 1. Search terms.

	→ AND	
→OR	cerebral autoregulation	TFA
	cerebral homeostasis	transfer function
	cerebral blood flow autoregulation	transfer-function
	cerebral pressure regulation	transfer analysis
	cerebral pressure autoregulation	
	cerebral blood pressure regulation	
	cerebral blood pressure autoregulation	
	cerebrovascular autoregulation	

Transfer function analysis

Evaluation of CA by transfer function analysis is based on the concept that CA minimizes the effect of spontaneous BP oscillations on CBFV. Without CA each spontaneous oscillation in BP would cause an oscillation of a similar duration, magnitude and frequency in CBFV. The method of transfer function analysis has already been used extensively in, for example, the investigation of cardiovascular control, respiratory sinus arrhythmia and renal auto-regulation [43-45]. Spectral analysis, such as performed with the fast Fourier transform (FFT), transforms time series of BP and CBFV to the frequency domain. Then, the transfer function between the two signals can be calculated as:

$$H(f) = S_{xy}(f) / S_{xx}(f) \qquad 1.$$

Where $S_{xx}(f)$ is the autospectrum of the input signal, BP, and $S_{xy}(f)$ is the cross spectrum between the input signal, BP, and output signal, CBFV [7]. With the transfer function the associated relative power (gain) and timing (phase) can be described using the real part $H_R(f)$ and the imaginary part $H_I(f)$ of the complex transfer function:

$$\text{gain: } |H(f)| = \sqrt{\{|H_R(f)|^2 + |H_I(f)|^2\}} \qquad 2.$$

$$\text{phase: } \Phi(f) = \tan^{-1}[H_I(f) / H_R(f)] \qquad 3.$$

An estimate of reliability of the relationship between the two signals can be found as the squared coherence:

$$\text{coherence: } MSC(f) = |S_{xy}(f)|^2 / [S_{xx}(f)\, S_{yy}(f)] \qquad 4.$$

where $S_{yy}(f)$ is the autospectrum of changes in CBFV.

As representative of the linear association between the fluctuation in blood pressure and cerebral blood flow, the coherence is in some studies also used as a measure for CA. Coherence approaching zero indicates no relationship between BP and CBFV, whereas a coherence approaching unity suggests a linear relationship indicating CA impairment.

In the first study of CA using the transfer function approach and spontaneous fluctuations in BP estimates of the amplitude frequency response (gain) and coherence were obtained, but not the phase frequency response [39]. The studies following after the first publication of

Giller et al. all reported the transfer function in different ways, some calculating the gain and / or phase, and / or coherence, others using the transfer function to obtain the impulse and step responses from which autoregulation can be quantified using the autoregulation index or other measures [6, 28].

Different settings of transfer function analysis

Despite the fact that the quantification of the transfer function seems straight forward, many different settings are used, such as the type of input data (CBFV, ABP, raw- or averaged over heart-beats), sampling frequency, detrending, normalization, interpolation, filtering, anti-leakage window, window length, superposition and spectral smoothing. Input data stands for the data that is used as input for the transfer function analysis, i.e. beat-to-beat BP in mmHg and CBFV in cm/s. The sampling frequency defines the number of times per second that the continuous signals are sampled and used for analysis. Some studies resampled their signals after storage, in those cases the resampling frequency is taken as sampling frequency for this review. Resampling is for example used to obtain equidistant time interval data for beat-to-beat data. Detrending, normalization, interpolation and filtering are four examples of pre-processing steps. Detrending means removing any linear or non-linear trends from the input data to avoid distortion of the low-frequency power. Normalization of BP and / or CBFV can be used to account for some inter-subject variability. An example of normalization is dividing the signal by its mean value, resulting in zero-mean signals which reflect relative changes in BP and/or CBFV [6]. Interpolation, a method of constructing new data points within the range of a discrete set of known data points, can be used to create equidistant time intervals (in case of beat-to-beat data) which is a prerequisite for transfer function analysis. Interpolation can also be used to downsample the data. Care must be taken to apply anti-alias filtering before downsampling. Filtering can be used to delete frequencies that are of no interest, such as very high frequencies or extremely low frequencies. For the estimation of the transfer function, the signal needs to be broken into overlapping segments (windows), to reduce the random errors in the estimates. However, this also leads to a distortion known as spectral leakage [57]. There are different kinds of anti-leakage windows, such as cosine-tapered window and Hanning window [58]. The window length represents the number of data points in that window, which will then define the frequency resolution of the transfer function estimates. Estimation errors in transfer function analysis are then reduced by averaging auto and cross-spectral estimates over the multiple data segments (Welch method). Further improvements can be achieved by spectral smoothing, by applying a low-pass filter to spectral estimates, before calculating the transfer function with equation 1

above. Usually triangular moving average filters are used for this purpose.

Statistics

The results of the articles were combined to obtain reference values for CA for all units used in the literature for calculating the transfer function. To obtain normal values for CA from the reported results, all the retrieved values were combined. The pooled mean and pooled standard deviation are calculated by weighting the individual values with the sample size of the corresponding study.

RESULTS

One hundred and ninety publications met the search criteria and were evaluated. Figure 1 shows a flowchart of the literature review process. After reading abstracts and, if necessary, whole articles, 85 publications were excluded because the studies were performed in animals or they used any manoeuvre or intervention to quantify CA. By means of the snowball method four articles were added to the set of included articles. Hence, 113 publications were eligible for review. Table 2 lists the set of included publications. The term dynamic CA was introduced by Aaslid in 1989 [67] and the transfer function analysis was first used to quantify CA by Giller et al. in 1990 [39], therefore the articles included in this review were all published between 1990 and 2012 (Figure 2). Figure 2 shows the number of studies using spontaneous oscillations in BP and CBFV to assess dynamic CA for each year. Study details are discussed in the next sections.

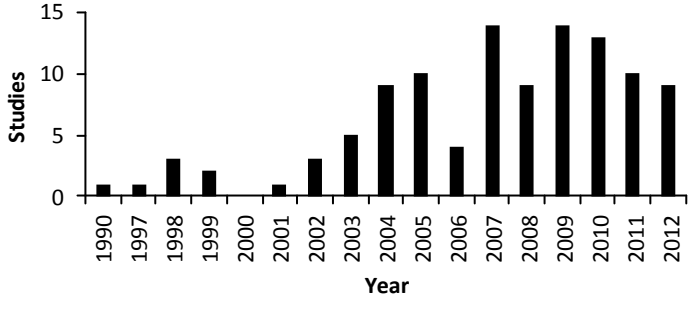

Figure 2. Number of included studies plotted against the year of publication.

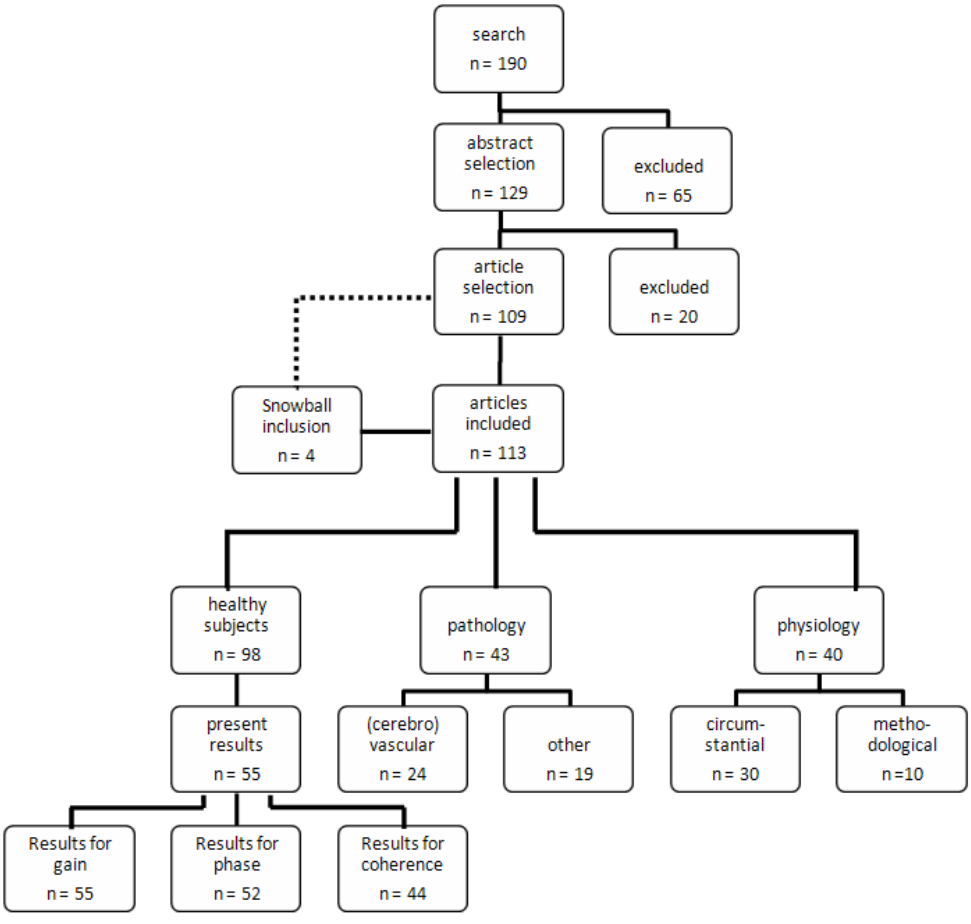

Figure 1. Study inclusion diagram.

A total of 85 (65+20) publications were excluded because they were animal studies or studies that used any manoeuvre or intervention to quantify CA. Through the snowball method (dashed line) four articles were added. All included publications were categorized in one or more groups: healthy subjects, pathology and/or physiology. The group 'healthy subjects' includes studies which used healthy subjects for their analysis. A subgroup is created for the studies which presented the numeric values for their CA results in their articles (box: present results). The group 'Pathology' includes studies which investigated CA in a pathological condition, further categorized as '(cerebro) vascular disease' and 'other'. The group 'Physiology' includes studies which obtained CA values in physiological situations, such as hypercapnia and exercise. This group is further categorized as 'circumstantial situations' and those investigating methodological issues (box: 'methodological') . *n* is the number of studies found.

Table 2. Summary of the literature review of studies which included healthy subjects.

Reference	Year	n healthy subjects
Ainslie et al. [1]	2007	14
Ainslie et al. [4]	2007	5
Ainslie et al. [7]	2008	10
Ainslie et al. [18]	2008	10
Ainslie et al. [27]	2012	10
Ainslie et al. [31]	2008	10
Bailey et al. [34]	2009	9
Bailey et al. [40]	2012	12
Bellapart et al. [46]	2011	5
Blaber et al. [10]	1997	8
Brassard et al. [51]	2012	10
Brodie et al. [54]	2009	10
Carrera et al. [59]	2009	18
Chen et al. [62]	2006	11
Claassen et al. [37]	2009	8
Claassen et al. [68]	2009	8
Cooke et al. [71]	2004	8
Cooke et al. [77]	2006	7
Cooke et al. [81]	2003	10
Deegan et al. [73]	2011	45
Deegan et al. [91]	2011	544

Reference	Year	n healthy subjects
Hilz et al. [2]	2004	24
Hu et al. [5]	1999	37
Immink et al. [9]	2005	10
Iwasaki et al. [22]	2007	6
Iwasaki et al. [29]	2012	15
Iwasaki et al. [32]	2007	15
Iwasaki et al. [38]	2011	11
Jachan et al. [41]	2009	0
Jochum et al. [47]	2010	20
Kim et al. [49]	2008	10
Kim et al. [52]	2007	7
Kim et al. [55]	2009	10
Kuo et al. [60]	1998	33
Lind-Holst et al. [63]	2011	9
Lorenz et al. [65]	2009	41
Low et al. [69]	2009	9
Marmarelis et al. [76]	2012	12
Marthol et al. [78]	2005	23
Marthol et al. [82]	2007	15
Muller et al. [89]	2003	42
Muller et al. [72]	2003	33

Reference	Year	n healthy subjects
Ogoh et al. [3]	2007	7
Panerai et al. [6]	1999	47
Panerai et al [17]	1999	16
Panerai et al. [24]	2002	0
Panerai et al. [30]	2004	0
Panerai et al. [33]	2005	14
Panerai et al. [28]	1998	0
Peng et al. [42]	2008	13
Peng et al. [48]	2010	13
Porta et al. [50]	2008	61
Purkayastha et al. [53]	2012	10
Ramos et al. [56]	2006	0
Reinhard et al. [61]	2003	0
Reinhard et al. [64]	2003	0
Reinhard et al. [66]	2003	0
Reinhard et al. [70]	2005	25
Reinhard et al. [26]	2003	0
Reinhard et al. [80]	2004	0
Reinhard et al. [84]	2007	94
Sakagami et al. [90]	2011	38
Sammons et al.[92]	2007	0

Table 2. (Continuation) Summary of the literature review of studies which included healthy subjects.

Reference	Year	n healthy subjects
Dutsch et al. [93]	2004	0
Eames et al. [96]	2004	14
Eames et al. [99]	2005	1
Edwards et al. [88]	2002	9
Fisher et al. [83]	2008	9
Formes et al. [107]	2010	10
Fritzsch et al. [100]	2010	6
Fu et al. [113]	2005	15
Gelinas et al. [85]	2012	21
Giller et al. [39]	1990	1
Gisolf et al. [125]	2002	12
Gommer et al. [128]	2012	20
Gommer et al. [112]	2010	19
Gommer et al. [132]	2008	0
Hamner et al. [134]	2004	9
Haubrich et al. [137]	2010	25
Haubrich et al. [139]	2004	30

Reference	Year	n healthy subjects
Muller et al. [94]	2005	33
Murrell et al. [97]	2007	9
Nakagawa et al. [102]	2009	65
Nakagawa et al. [74]	2011	23
Narayanan et al. [105]	2001	10
Nishimura et al. [108]	2010	13
Nishimura et al. [110]	2007	8
Ocon et al. [114]	2009	7
Ogawa et al. [116]	2010	10
Ogawa et al. [123]	2008	14
Ogawa et al. [126]	2009	14
Ogawa et al. [129]	2007	12
Ogawa et al. [131]	2006	11
Ogoh et al. [133]	2005	7
Ogoh et al. [135]	2007	8
Ogoh et al. [138]	2005	7
Ogoh et al. [140]	2005	7

Reference	Year	n healthy subjects
Serrador et al. [95]	2005	22
Subudhi et al. [98]	2011	29
Subudhi et al. [103]	2009	12
Subudhi et al. [104]	2010	28
Ter Laan et al. [106]	2011	0
Tzeng et al. [109]	2012	105
Tzeng et al. [111]	2010	19
van Beek et al. [79]	2010	27
Wang et al. [121]	2005	10
Wilson et al. [124]	2010	14
Zhang et al. [127]	2009	13
Zhang et al. [130]	2004	8
Zhang et al. [101]	2007	9
Zhang et al. [87]	1998	10
Zhang et al. [136]	2002	12
Zhu et al. [75]	2011	6

Variation in transfer function analysis parameter settings

42

Figure 3 shows the overall heterogeneity in the use of transfer function of the studies. The percentage of studies (y-axis) using specific settings is shown for the ten most reported analysis parameters (x-axis): input data (beat-to-beat values or raw instantaneous CBFV and BP), sampling frequency, detrending, normalization, interpolation, filtering, anti-leakage window, window length, superposition, and spectral smoothing. It should be noted that not every study reported information on all of the ten analysis parameters. It is possible that some of the investigators thought no application of those were general and therefore not necessary to mention.

As input data, the majority of the studies have used beat-to-beat BP and CBFV for their analyses (75 %), 23 % used the raw waveform data and 2 % did not report their setting. Regarding the sampling frequency, studies reported a wide range in different sample frequencies: the lowest sampling frequency was 0.5 Hz [72], the highest 100 Hz [73-75], whereas 28 % of the studies did not report sampling frequency.

The vast majority of studies (85 %) did not report whether or not they had used detrending. When detrending was mentioned, 3 % did not specify the method used, 7 % used linear detrending, and 5 % applied non-linear functions to detrend the data.

Regarding normalization of CBFV, 9 % of the included studies normalized the CBFV, 6 % normalized both BP and CBFV, and 84 % of the studies reported no normalization of the data.

The different interpolation methods used by the studies can roughly be split up into two groups: linear- (32 %) or polynomial / spline (24 %) interpolation. Again, a substantial part of the studies (43 %) did not report whether or not they had used interpolation or not.

Regarding filtering, 89 % did not mention using a filter, 7 % used low pass filtering, 2 % high pass filtering and 2 % used other types of filtering.

Three types of anti-leakage windows were used: the Hamming or Hanning window (27 %), the cosine-tapered window (5 %) and the rectangular window (1 %).

The window length was not reported in 68 % of the studies. The other 32 % of the studies showed a high diversity in window lengths ranging from 30 s to 256 s. Superposition was only mentioned in 31 % of the studies; most of those studies (23 %) used 50 % superposition.

Spectral smoothing was only reported in 4 % of the studies.

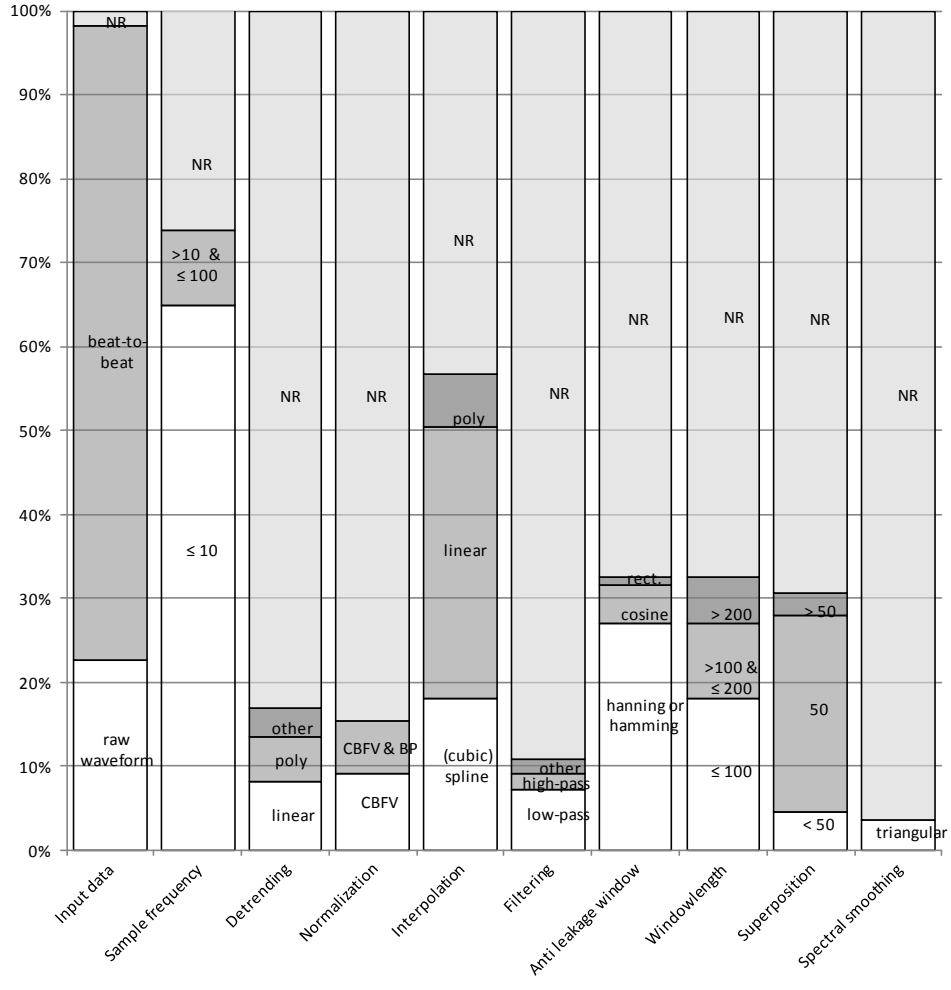

Figure 3. The percentage of studies (y-axis) using specific settings for the quantification of the transfer function for the ten most reported analysis parameters (x-axis): input data, sampling frequency, detrending, normalization, interpolation, filtering, anti-leakage window, window length, superposition, spectral smoothing. NR is not reported, rect. is rectangular, poly. is polynomial.

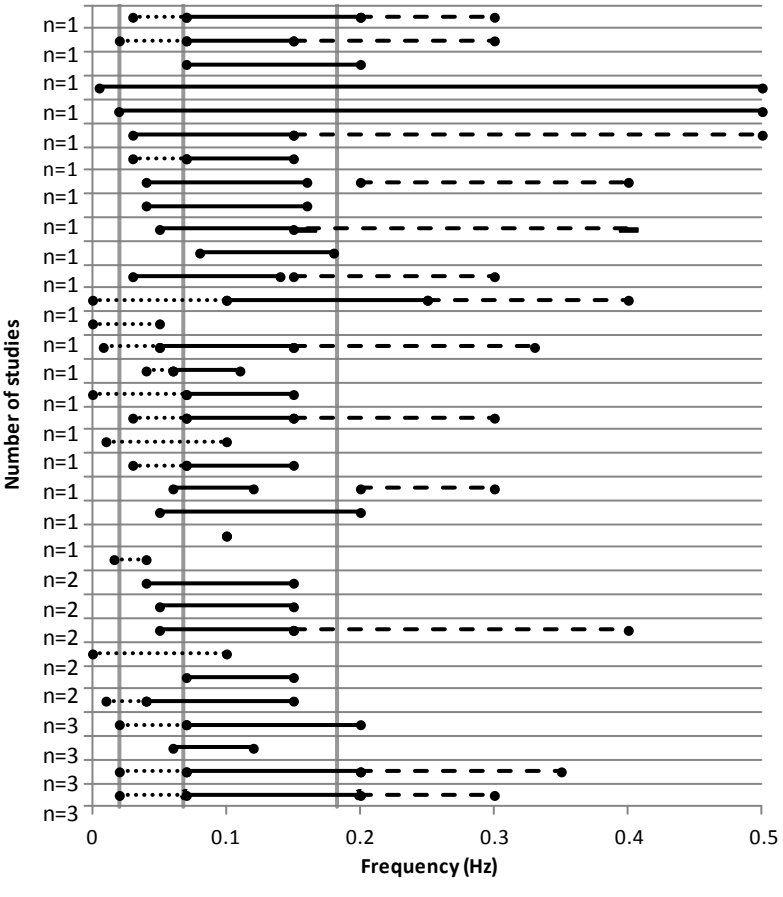

Figure 4. Overview of the frequency distributions used in the different studies.

The vertical axis represents the number of studies (n) who used the corresponding frequency bands. The dotted lines show frequency distributions used to represent the very low frequency range, the solid lines represent the low frequency range, and the dashed lines represent the high frequency range. The grey vertical lines indicate the average cut-off start frequency used in the studies for the very low-, low-, and high-frequency range, respectively.

Results of transfer functions analysis

The transfer function analysis results in values of gain, phase and coherence. Since the transfer function contains frequency-specific information the gain, phase and coherence can be represented in graphs or as values for different frequency bands (using the mean, the integrated area or the maximal values). Figure 4 gives an overview of the frequency ranges as they were reported in the retrieved studies. A total of 92 studies reported their frequency ranges.

Gain, phase and coherence results can be represented in different units, for example as cm/s/mmHg or %/% for gain. The units for gain used by the retrieved studies were cm/s/mmHg (64 %), %/% (15 %), %/mmHg (9 %), cm/s/% (2 %), dB (4 %), unit/mmHg (2 %) and no units (n.u.) (4 %). For the phase the following units were used: degrees (31 %), radians (69 %) and n.u. (2 %). For the calculation of reference values for phase in this study, degrees were converted to radians.

Reference values

From the search, 55 studies, with a total of 958 subjects, were included containing values for gain, phase and / or coherence of healthy adults. Concerning the differences in frequency ranges used, the data were sorted for very low frequency (VLF), low frequency (LF), and high frequency (HF) using the denominations as they were used in the articles. For example, Van Beek et al. used the frequency range 0.02 - 0.07 to represent the VLF, 0.07 - 0.2 to represent the LF, and 0.2 - 0.35 to represent the HF [79], consistent with the ranges proposed by Zhang et al. [35]. Reference values for the non-invasive determination of CA with transfer function analysis in healthy subjects are given in Table 3. In Figure 5 a graphical representation of the transfer function results for healthy subjects is given for the gain in cm/s/mmHg, the phase in radians and the coherence. The graph was obtained by extrapolating the mean values and standard deviations as quantified by each study, over the corresponding frequency band. Next the pooled mean and standard deviation for all the studies were calculated for each frequency point.

Distinction of CA in physiology

CA studies have been performed in different physiological conditions, such as hypercapnia [7], exercise [83], and head down tilt [85]. Hypercapnia, a model for impaired CA,

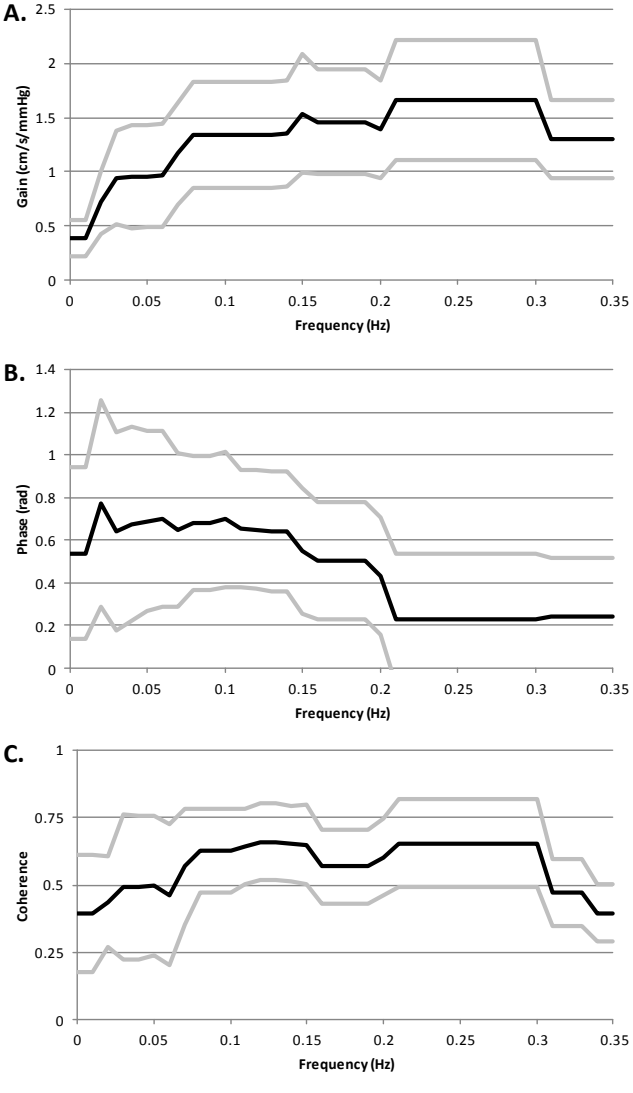

Figure 5. Graphical representation of the transfer function values found in the different studies.

The graph shows the gain in cm/s/mmHg (A), the phase in radians (B), and the coherence (C). Results are represented by the pooled mean (black line) ± pooled standard deviation (grey lines).

Table 3. Reference values for the non-invasive determination of CA with transfer function analysis in healthy adults. The values for gain are represented for 6 different indices, cm/s/mmHg, %/%, %/mmHg, dB, unit/mmHg, mmHg/cm/s, cm/s/%, and no units (n.u.), respectively. The phase is represented in radians.

Frequency domain measurements					
Frequency band	**Gain Units**	**n subjects**	**n studies**	**Value**	**Std**
VLF	cm/s/mmHg	422	23	0.77	0.34
	%/%	89	4	0.74	0.21
	%/mmHg	134	1	1.26	0.63
	dB	16	2	-0.43	1.14
	unit/mmHg	37	1	1.64	0.57
	mmHg/cm/s	0	0	-	-
	cm/s/%	8	1	0.36	0.05
	n.u.	30	1	1.59	0.72
LF	cm/s/mmHg	548	33	1.12	0.42
	%/%	83	5	1.63	0.5
	%/mmHg	232	4	1.46	0.4
	dB	16	2	2.95	0.85
	unit/mmHg	37	1	1.41	0.46
	mmHg/cm/s	7	1	0.7	0.1
	cm/s/%	8	1	0.45	0.03
	n.u.	60	3	1.64	0.48
HF	cm/s/mmHg	299	24	1.33	0.43
	%/%	0	0	-	-
	%/mmHg	88	2	1.36	0.15
	dB	0	0	-	-
	unit/mmHg	37	1	1.26	0.48
	mmHg/cm/s	7	1	0.8	0.1
	cm/s/%	8	1	0.3	0.04
	n.u.	30	1	1.9	0.43
	Phase Units	**n subjects**	**n studies**	**Value**	**Std**
VLF	radians	573	31	0.79	0.5
LF	radians	781	41	0.61	0.27
HF	radians	409	27	0.05	0.28
Frequency band	**Coherence Units**	**n subjects**	**n studies**	**Value**	**Std**
VLF	n.u.	799	29	0.49	0.16
LF	n.u.	834	35	0.67	0.15
HF	n.u.	558	25	0.44	0.17

is the most investigated physiological state in CA studies. During CO_2 inhalation, elevations in PCO_2 lead to vasodilatation of cerebral arterioles in the downstream bed and subsequently to an increase in cerebral blood flow [86]. This strong vasodilatation could impair the vasoconstrictive response to a blood pressure increase. Likewise, it could impair further dilatation in response to blood pressure decrease. These changes can result in lower phase and in an increased gain because there is less efficient damping of the effect of blood pressure fluctuations on the cerebral blood flow. However, the increased blood flow due to CO_2 will also increase blood flow velocity, and unless relative flow-velocity changes are used to calculate gain, this higher absolute flow velocity will lead to higher gain estimates. In this case, a higher gain would not necessarily reflect impaired autoregulation. It is therefore important to consider changes in phase and gain together. This review has found four studies reporting results for CA during hypercapnia [7, 17, 87, 88]. Zhang et al. tested the hypothesis that hypercapnia impairs CA, by giving a gas mixture of 5 % CO_2 and 21% O_2 balanced with N_2 to their subjects [87]. They have found that transfer gain and coherence increased and phase decreased in the frequency range 0.07 - 0.20 Hz compared with baseline, suggesting an impairment of CA during hypercapnia. Edwards et al. investigated the effect of altered arterial PCO_2 on CA [88]. Panerai et al. made recordings before, during and after breathing a mixture of 5 % CO_2 in air [17]. During 5 % CO_2, the coherence and the gain were significantly increased for frequencies below 0.05 Hz and the phase was reduced for the frequency range 0.02 - 0.1 Hz. Ainslie et al. achieved incremental hypercapnia in 10 healthy male subjects through 4 - min administration of 4 % and 8 % CO_2 [7]. In this study, hypercapnia caused a progressive increase in PCO_2, but there were no evident changes in transfer function gain or coherence. However, the phase in the VLF range was reduced during the most severe level of hypercapnia. The results of the four studies for the gain, phase, and coherence are plotted against the averaged reference value graph for healthy subjects, quantified using the 55 studies as described above, in Figure 6. While the four hypercapnia studies reported significant differences in CA gain, phase, and/or coherence between normal healthy state and hypercapnia, no evident differences are visible between the results of the hypercapnic studies and the calculated reference values of this review.

Assessment of CA in pathophysiology

CA studies using transfer function analysis are performed in specific clinical conditions (n = 43). This review showed that of the clinical studies, most were done in patients with carotid artery disease (n = 5) [5, 64, 66, 80, 100]. Patients with carotid stenosis may have several factors that affect the outcome of TFA of cerebral haemodynamics. First,

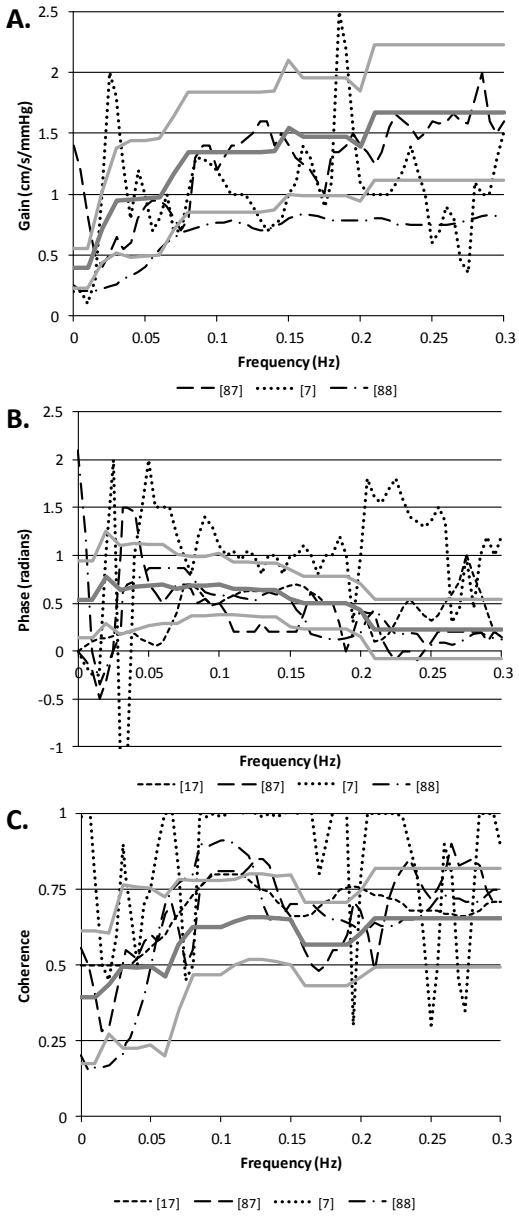

Figure 6. Graphical representation of the results found by the studies investigating the effect of hypercapnia on CA plotted against the average normal values (grey lines represent the pooled standard error of the mean). The average normal values are quantified using the 55 studies also presented as the area between the grey lines in Figure 5. The graph shows the gain in cm/s/mmHg (A), the phase in radians (B) and the coherence (C). Results are represented by the pooled mean for each study. The different studies are represented by the black lines.

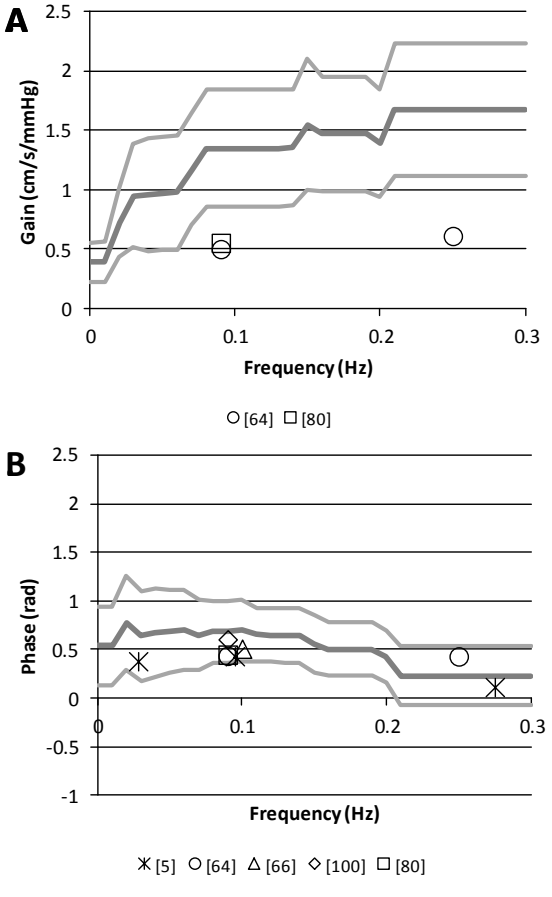

Figure 7. Graphical representation of the results found by the studies investigating the effect of stenosis on CA plotted against the average normal values (grey lines represent the mean ± pooled standard deviation). The average normal values are quantified using the 55 studies also presented as the shaded area in Figure 5. The graph shows the gain in cm/s/mmHg (A) and the phase in radians (B). Results are represented by the pooled mean for stenosis of at least 50 %. The different studies are represented by different symbols (◇, □, △, ○, ✳).

these patients may have higher systemic and cerebral vascular resistance (due to chronic hypertension that is often seen in such patients) and they may have increased vascular stiffness due to the combination of hypertension, vascular disease and aging. The increased cerebrovascular resistance could enhance effective damping of rises in BP. This could lead to lower gain values. Lower gain in hypertension was indeed observed by Zhang et al. [101]. With regard to the effects of the stenosis, there is post-stenotic vasodilatation (to compensate for the upstream stenosis). These changes may result in a lowered vasodilatory capacity, which could affect gain and phase values measured in the affected circulation. However, carotid stenosis may also lead to lower (post-stenotic) cerebral blood-flow velocity, and when absolute values are calculated for gain, this may lead to lower gain estimates that do not necessarily reflect autoregulation. Fritzsch et al. examined whether stenotic disease of the carotid affects both neurovascular coupling and CA [100]. They studied 10 patients with altogether 13 stenosed arteries (≥ 50 %) and found no relevant differences between controls, non stenosed sides and stenosed sides. In this group of patients with mainly moderate stenosis, CA seemed to be unaltered. Hu et al. enrolled eighty-three consecutive patients with various degrees of carotid stenosis [5] and found that patients with unilateral high-grade (greater than 90 % stenosis) carotid stenosis demonstrated significant reduction in LF phase angle and HF gain. Reinhard et al. investigated 168 patients with severe carotid stenosis or occlusion using the transfer function analysis for CA [64], 30 patients with severe bilateral carotid stenosis (≥ 75 %) or occlusion [66], and 58 patients with severe unilateral stenosis undergoing carotid endarterectomy or stenting [80]. They found that all CA parameters were clearly impaired ipsilateral to the stenosis, compared with contralateral sides.

The results of these studies (for stenoses of 50 % and higher) for the phase and gain are plotted against the averaged reference value graph for healthy subjects in Figure 7.

DISCUSSION

Although disorders of CA may lead to unfavourable clinical outcomes, there is currently no standardized test of autoregulation that may be performed safely and easily in a wide sphere of clinical conditions. Non-invasive methods for the quantification of CA have been developed, but a gold standard is lacking. The objective of our investigation was to provide an overview of transfer function techniques used in the assessment of dynamic CA while using spontaneous oscillations. Our main findings are that a large number of studies have used transfer function analysis to quantify CA – indicating the importance of having a non-invasive method to quantify CA. One might have expected, from the popularity and wide-spread use

of this method, and from this large amount of published studies, that issues of methodological variation had been properly addressed. In contrast, we found that a strong diversity exists in transfer function analysis, that no standard protocol exists, and that most studies fail to report important settings that were used to calculate the transfer function.

The high diversity in the implementation of the transfer function reported in this review, infers that it is difficult to replicate or compare the results of the different studies. A major cause of the broad diversity is the difference in frequency range definitions. Dynamic CA is a frequency dependent phenomenon, and transfer function analysis actually makes use of this by quantifying frequency dependent changes in phase and gain. Therefore, it is obvious that changing the frequency definitions in a study prohibits direct comparison of that study's results with those using other definitions. This makes it all the more surprising, if not worrisome, that we encountered such wide variety in frequency ranges in this field.

Several other factors also contribute to this diversity, for example the differences in data pre-processing, such as detrending, normalization, interpolation and filtering. It is not yet known what effect the use of those different settings has on the outcome of transfer function analysis. Gommer et al. compared raw data pre-processing by mean subtraction with smoothness priors detrending [112]. They found no significant difference between these settings. However, the effect of other settings, for example the type of anti-leakage window used, have not yet been studied.

Despite this broad diversity in CA values, pooling the data of the different studies led to encouraging results. Even though there was the expectedly large between-centre variability, the pooled data revealed multi-centre frequency plot trends for phase and gain that were consistent with the original high pass filter model for dynamic CA [35], with higher values for phase in the lower frequencies, decreasing with increasing frequency, and lower values for gain in the lower frequencies, increasing with higher frequency.

The results of CA in the pathophysiological condition of carotid artery stenosis show that, in most cases, phase is below average but remains within one standard deviation of values for healthy controls, implicating that it is difficult to distinguish normal from impaired CA due to stenosis. However, gain differences do exceed this one standard deviation limit, suggesting that gain could be a possible parameter to distinguish between impaired CA due to stenosis and normal CA. The physiological condition of hypercapnia showed that only for the coherence around 0.1 Hz all studies differed more than one standard deviation from

reference values. However, for gain, phase and coherence a large inter-study variability was found, causing high standard deviations which make that a result needs to lie further from the average reference value before it can be indicated as aberrant. Overall, for hypercapnia and carotid stenosis it was difficult to distinguish between normal and impaired CA. However, a limitation of this comparison of individual studies with large population averages is that different subgroups of data of a variety of populations and collected in different conditions are compared. This may indicate that results are incompatible between different estimators. Still, we envisage that if we succeed in standardizing transfer function analysis, this will make it possible to have less dispersion in the normal values for CA, and therefore make it easier to distinguish between normal and impaired CA.

Regarding the possibility of different transfer function analysis settings causing the diversity in results, there are also other possible causes that should be considered. An important factor to consider in transfer function analysis is the potential presence of non-stationarities in the data, which could lead to distorted results. Rigorously, CA is a non-linear phenomenon due to the modulation of cerebrovascular resistance. Next to the potential presence of non-stationarities, other physiological variables can affect the blood flow and its regulation, such as PCO_2 [115], brain metabolic activity, haematrocrit, and sympathetic tone [117]. Despite demonstration that for small changes in BP and CBFV, as observed during spontaneous fluctuations, the linear model provides an acceptable approximation [33], it is possible that non-stationarities cause a high spread in the results.

Furthermore, all studies measured CBFV in the middle cerebral artery (MCA) using TCD, rather than cerebral blood flow. Changes in CBFV reflect changes in cerebral blood flow only if the MCA diameter remains constant. Numerous studies have shown that MCA diameter in humans remains relatively constant under a variety of haemodynamic conditions [118-120]. However, there is a possibility that the measurement quality differs between the centres [65], leading to a higher spread in transfer function outcomes.

The outcome and conclusion of this review raises several important research questions: first what is the reason for the fact that different centres apply transfer function in different ways? An explanation could be the misinterpretation and insufficient documentation concerning the settings for transfer function analysis in previous studies.
Second, how is the outcome of the transfer function influenced by different settings? To answer this question a multi-centre study is needed, comparing the results of different centre methodologies for transfer function analysis all analysing the same datasets [122].

Third, is there a method to differentiate between intact and impaired CA? In the literature, numerous ways of differentiating between impaired and intact CA are presented, but it is unknown which one is most robust. In this review, we only focused on the separate outcomes for gain, phase and coherence for the separate frequency bands, instead of looking at a method which particularly focuses on the differentiation between intact and impaired CA, i.e. by combining the gain, phase, and coherence value.

The fourth question is whether the transfer function analysis is the optimal way to quantify CA, regardless of the fact that it is the most widely used method. Possibly, other methods, including non-linear alternatives, could provide better results. A study comparing the different methodologies is therefore also necessary.

The fifth and maybe most important question is when do we speak of impaired CA. In pathological cases, such as carotid stenosis and brain injury, changes in cerebrovascular function are often confirmed with a complementary method of determining cerebral haemodynamics, i.e. CO_2 reactivity. However, no generally accepted gold standard test for CA exists, making it difficult to ascertain whether a change in transfer function parameters reflects a better or worse CA. In addition, at this moment, no straightforward definition of impairment of CA has been reported. Studying the basics of CA and the effects of changes in CA on transfer function parameters is therefore of great necessity for the valid interpretation of results.

Overall, despite considerable interest and urgency to bring autoregulation testing to the clinical arena, the overall heterogeneity of the studies, as reflected in this review, calls for standardization of the methodology of CA quantification. With the standardization of the methodology it may be possible to increase the reliability of the CA measurements, making it possible to create gold standard values for CA in healthy subjects. These normative values will make it possible to distinguish between normal and abnormal CA. Ultimately this may lead to international guidelines for the interpretation of CA indices, making it possible to start international multi-centre studies to determine the predictive clinical value. Furthermore, these guidelines for the interpretation of CA indices will make it possible to monitor patients CA in a standard manner. Such monitoring might help for example to identify patients at risk for ischemic events, but could also help optimizing BP management in uncontrolled hypertensive patients.

In summary, there is a high need for extensive standardization and validation of the CA quantification method to improve the reliability and usefulness of CA in clinical practice.

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Editor's comment:

This paper is a laudable joint initiative by a group of researchers in the Cerebral Autoregulation Research Network (CARNnet -http://www.car-net.org/) to assess one of the most commonly used techniques for the measurement of autoregulation (Transfer Function Analysis). Different groups internationally use slightly different variants of the methods, and this paper addresses the justified concern that small differences in analysis technique may be a confounding factor in comparing results of different studies. Considerable dispersion was indeed observed in results of analysing identical datasets in different groups (though inferences as to impairment of autoregulation were generally consistent). Of additional concern is the observation that even when all groups were using supposedly identical methods, results differed considerably. This highlights the need for quality control, more training and multidisciplinary collaboration to improve robustness of results and compatibility between studies, a lesson one hopes might be taken up by the wider research community.

Richard Black, Editor in Chief

Chapter 3.

Between-centre variability in transfer function analysis: the CARNet study

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ABSTRACT

Transfer function analysis (TFA) is a frequently used method to assess dynamic cerebral autoregulation (CA) using spontaneous oscillations in blood pressure (BP) and cerebral blood flow velocity (CBFV). However, controversies and variations exist in how research groups utilise TFA, causing high variability in interpretation. The objective of this study was to evaluate between-centre variability in TFA outcome metrics. For this study, 15 centres analysed the same 70 BP and CBFV datasets from healthy subjects (n = 50 rest; n = 20 during hypercapnia); 10 additional datasets were computer-generated. Each centre used their in-house TFA methods; however, certain parameters were specified to reduce a priori between-centre variability. Hypercapnia was used to assess discriminatory performance and synthetic data to evaluate effects of parameter settings. Results were analysed using the Mann-Whitney test and logistic regression. A large non-homogeneous variation was found in TFA outcome metrics between the centres. Logistic regression demonstrated that 11 centres were able to distinguish between normal and impaired CA with an AUC > 0.85. Further analysis identified TFA settings that are associated with large variation in outcome measures.

These results indicate the need for standardisation of TFA settings in order to reduce between-centre variability and to allow accurate comparison between studies. Suggestions on optimal signal processing methods are proposed.

INTRODUCTION

Oscillations in arterial blood pressure (BP) and cerebral blood flow velocity (CBFV) can be used for non-invasive assessment of the dynamic pressure-flow relationship of the cerebral circulation. Dynamic cerebral autoregulation (CA) is an important determinant of this pressure-flow relationship, and transfer function analysis (TFA) is a widely used linear approach to quantify CA.

The transfer function method was first carried out to quantify dynamic CA by Giller et al. [1] analysing the relationship between oscillations in BP and CBFV in the frequency domain. Evaluation of CA by TFA is based on the concept that CA minimises the effect of dynamic BP fluctuations on CBFV, which is reflected by reduced low-frequency gain and phase-lead of CBFV over BP. Without CA, CBFV would passively follow BP, and TFA would show constant gain and zero phase across the low-frequency band. TFA has been employed to obtain quantification of CA during spontaneous oscillations in BP and CBFV. Using spontaneous oscillations to assess CA is often preferable to interventions which induce (larger) BP fluctuations, since these interventions require patient cooperation and/or can be uncomfortable; making them unsuitable in cases of severe illness or illness that reduces mobility. As a consequence, TFA is currently a widely used method in literature to quantify CA, both in basic physiological research and in clinical studies. However, there are large differences in TFA outcomes between studies. These differences may be due to large inter-subject variation in CA, even for healthy controls, but may also be due to methodological differences between centres.

A systematic review on TFA found that most studies have used relatively small sample sizes, and were all done under different experimental conditions and for diverse research purposes [2]. Furthermore, this review showed a marked diversity in the signal processing methods used for TFA. This makes it difficult, if not impossible, to replicate or compare the results of the different studies or obtain normal values for healthy subjects.

The objective of this multi-centre study was to evaluate the differences in transfer function gain, phase and coherence outcomes when the same datasets are analysed by different centres using their corresponding routine signal processing methods for TFA. Our study also investigated the effects of different parameter settings for the analysis on transfer function outcomes. Furthermore, our study evaluated the ability of TFA to identify the effects of hypercapnia [3].

MATERIALS AND METHODS

Multi-centre database

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Five different centres from the UK (authors DS, RB and MC), USA (author VN), and the Netherlands (author JC), provided datasets containing simultaneously recorded BP and CBFV of subjects during rest, to create a multi-centre database. Two centres also provided datasets recorded during 5 % CO_2 breathing, a model known to alter transfer function outcome measures. One centre provided artificial datasets, using synthetic CBFV signals obtained by applying the models proposed by Tiecks et al. [4] to BP signals randomly selected from the available dataset. This Tiecks model provides the autoregulatory index (ARI), ranging from 0 to 9; in normal subjects ARI is approximately 5 with a standard deviation of 1. Ten signals were generated, one for each autoregulation index (ARI), covering the full range from 'absent' (ARI = 0) to 'fastest' (ARI = 9) autoregulation. Since most centres use beat-to-beat data to analyse transfer function gain, phase, and coherence, in this study we chose to use beat-to-beat data only. The beat-to-beat data were created using peak detection to identify subsequent beats, obtaining average values for BP and CBFV (unilateral) for each heart beat, and storing this information together with the beat-to-beat interval. The consecutive beat-to-beat intervals were used to reconstruct a time series of 5 min. The time-series data were linearly interpolated and resampled at 10 Hz to create equidistant data sampling over time. In some datasets an alternative procedure, with low-pass filtering of the original data (cut-off frequency of 0.5 Hz, third order Butterworth filter applied in the forward and reverse direction for a zero-phase response) was used to generate the mean BP and CBFV signals.

In total, the database contained 80 datasets ('subjects'), 50 beat-to-beat baseline datasets (normal resting baseline conditions); 20 5 % CO_2-breathing datasets (from 20 subjects who were also included in the baseline dataset); and 10 synthetic datasets. Data files were blinded to the analysing centres and presented in a randomised order.

Participating centres

The database was analysed by 15 centres and they returned their transfer function results to a central collecting site (AM, JC). These centres are included in the author affiliation section.

Methods

Centre routine

All centres were first asked to report their normal routine settings for TFA in a predefined spreadsheet, including sampling rate, filtering or other pre-processing steps, and details on how the TFA was performed (e.g., type of window, window length, number of windows).

Pre-specified settings for analysis

Centres were asked to analyse the data using their own normal routine, however with some restrictions. This decision was made based on a review [2] as well as a pilot study that had revealed such profound between-centre differences in TFA settings (for example different frequency band definitions, different units) that comparison between centres was impossible. Therefore, centres were requested to analyse the datasets using the following pre-specified settings: mean value beat-to-beat data as input data, no detrending, no prefiltering, number of windows set at 5, window length ranging between 90 s and 100 s with 50% superposition, Hanning window as anti-leakage window, no spectral smoothing and no phase unwrapping. Centres were asked to report TFA parameter results as mean values, using standardised units for phase (radians) and gain (cm/s/mmHg) and apply pre-specified frequency band definitions (very low frequency (VLF): 0.02 - 0.07 Hz; low frequency (LF): 0.07 - 0.15 Hz; high frequency (HF): 0.15 - 0.4 Hz). Every centre reported their TFA gain, phase and coherence values for each of the frequency bands for every dataset. To compare the transfer function data across the entire frequency spectrum, all centres were also asked to provide data to reconstruct frequency plots for gain, phase and coherence for ten specified subjects in the database.

Assessment of between-centre variability and comparison

As descriptors of between-centre variability, the median, quartile and min-max values for the TFA parameters generated by the 15 centres were listed, and clustering between centres for parameter outcomes was tested using three-dimensional plots for phase, gain and coherence for each frequency band.

Discriminatory power was assessed for each centre's method by comparing TFA parameter differences between normocapnia and hypercapnia. Finally, phase and gain values for each frequency band in the generated datasets were used as the reference standards to allow between-centre comparison; deviation from these reference values was used to create a ranking of the centres' method.

Effect of different transfer function settings

The usual routines, as reported by the different centres, were then evaluated. The routines contained different transfer function settings, such as sample frequency, interpolation, detrending, number of windows, window length, choice of anti-leakage window, percentage of superposition, and the definition of the frequency bands.

Explanation of these settings

The sample frequency defines the number of samples per second in which the continuous signals are stored. Interpolation can be used to create equidistant time intervals (in case of beat-to-beat data), by constructing new data points within the range of a discrete set of known data points. Most signal processing methods, including TFA, require equidistant data points. Detrending reduces the contribution of signal trends to the low-frequency power by removing any linear or non-linear trends from the input data. Pre-filtering can be used to delete frequencies that are of no interest, such as very high frequencies or extremely low frequencies. Data segments are usually windowed to reduce a particular kind of distortion called spectral leakage [5]. There are different kinds of anti-leakage windows, such as the cosine-tapered or Hanning window [6]. The window length defines the frequency resolution of the transfer function estimates. Averaging of spectral estimates over consecutive overlapped windows (Welch method) is used to reduce estimation errors (variance) in TFA estimates. As an alternative (or in addition), spectral smoothing can be achieved by applying a low-pass filter to spectral estimates, before calculating the transfer function. Triangular moving average filters are typically used for this purpose. Phase unwrapping is the inference of absolute phase from modulo-2π phase [7]. A cut-off value for the coherence can be used to assure statistical reliability of the transfer function. This cut-off value is influenced by the number of windows (the value is lower for a higher number of windows) and smoothing of the frequency spectra using for example triangular moving averaging [8].

To evaluate how differences in usual routine settings of the centres might influence the outcome of the TFA, the following settings were compared using the artificial (generated) datasets: sample frequency (1, 5, 10, 20 and 50 Hz), type of interpolation (linear and spline), type of detrending (none, removal of the mean, linear and third order polynomial detrending), number of windows (1, 3, 5 and 10), window length (25, 50, 100 and 300 s), type of anti-leakage window (Hanning, Hamming, rectangular and Tukey), and percentage window superposition (25, 50 and 75 %). Furthermore, different frequency bands were evaluated. For the VLF, the following two sets were evaluated: 0.01 - 0.04 Hz and 0.02 - 0.07 Hz. The LF was defined as 0.04 - 0.15 Hz, 0.07 - 0.15 Hz, and 0.07 - 0.2 Hz and the

HF as 0.15 - 0.4 Hz and 0.2 - 0.3 Hz. Since research has shown that some studies do not use three separate frequency bands [2], the outcomes for CA were also evaluated for 0.02 - 0.5 Hz.

For these comparisons, the actual values of gain and phase of the 10 ARI models were used as criterion standard. Next, using this same software, the effect of changing one of the settings as described above was investigated. The transfer function outcomes for VLF and LF gain and phase obtained for these different transfer function settings were compared by ranking the absolute deviation of the outcomes from the criterion standard values.

STATISTICS

Results are presented as median, min/max, and 1st and 3rd quartiles. Results were compared with the Mann-Whitney test. Logistic regression was used to construct a receiver operating characteristic (ROC) curve, in which the optimal sensitivity and specificity combination is visualised. The area under the curve (AUC) was quantified as a measure of quality of the classification model. A p-value < 0.05 was considered statistically significant.

RESULTS

Centre routine

Participating centres

The number of participating centres in this study was 15. The approach of centre 3 is distinct (Laguerre expansions [9]) from other frequency-domain approaches. Centre 14 did not report results for coherence. Table 1 and 2 (Supplement) give an overview of the normal routine settings of each centre and the settings used for this study, respectively. The centres differ in their signal processing methods for TFA in the following parameter settings: sample frequency, type of interpolation used, number of windows, window length, frequency resolution, type of spectral smoothing and phase unwrapping. Furthermore, centre 15 reported to normalise the BP and CBFV data by the mean value during the 5-min period and subtracted by 1 before applying TFA. Table 1 and 2 (Supplement) show that not all centres were able to report all their settings (not reported, NR) used for the TFA.

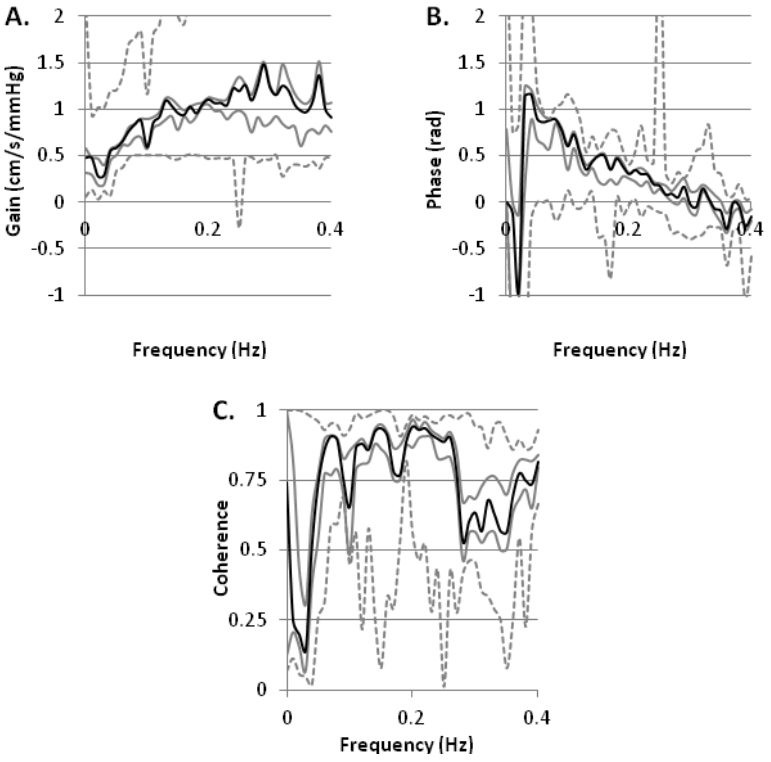

Figure 1. Average transfer function results (gain (A), phase (B) and coherence (C)) of the centres found for one subject during normocapnia. Results are represented as median (black line), min/max (grey dashed lines), and 1st/3rd quartile (grey solid lines) for the gain in cm/s/mmHg (top), phase in radians (middle),and coherence (bottom).

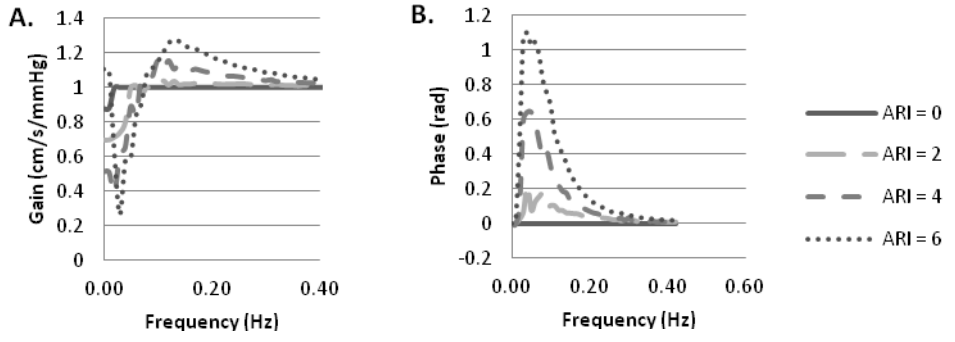

Figure 2. Transfer function gain (A) and phase (B) of the artificial datasets ARI 0, 2, 4 and 6, analysed using one set of parameter settings for TFA. The specified parameter settings were: sample frequency = 10 Hz, window length = 95 s, anti-leakage window = Hanning, number of windows = 5, percentage of superposition = 50%, frequency bands were VLF: 0.02 - 0.07Hz; LF: 0.07 - 0.15Hz; HF: 0.15 - 0.4Hz.

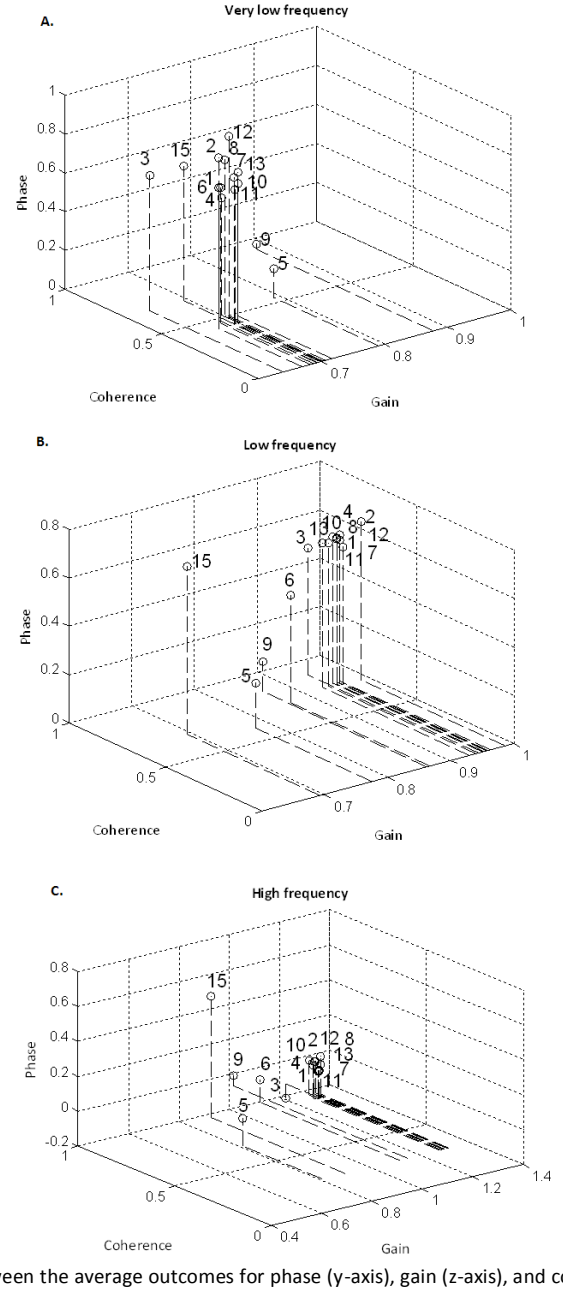

Figure 3. Relation between the average outcomes for phase (y-axis), gain (z-axis), and coherence (x-axis) of the centres for the very low frequency (A), low frequency (B), and high frequency (C). Vertical dashed lines indicate the projection of each point on the x-y plane. Centre 14 is excluded from the graph, because this centre did not report any values for the coherence.

Transfer function plots

All centres were asked to present frequency plots, analysed using the pre-specified settings, for certain datasets. Figure 1 represents the average transfer function plots generated by the 15 centres for a single subject under normocapnic baseline condition. These 15 plots are summarised as median (black line), 1st/3rd quartile (grey solid lines) and min/max (grey dashed lines). The transfer function plots for gain and phase of the generated datasets ARI = 0, 2, 4 and 6, generated when analysed using one set of parameter settings for TFA, are shown in Figure 2. The specified parameter settings were: sample frequency = 10 Hz, window length = 95 s, anti-leakage window = Hanning, number of windows = 5, percentage of superposition = 50%, frequency bands were VLF: 0.02 - 0.07 Hz; LF: 0.07 - 0.15Hz; HF: 0.15 - 0.4 Hz.

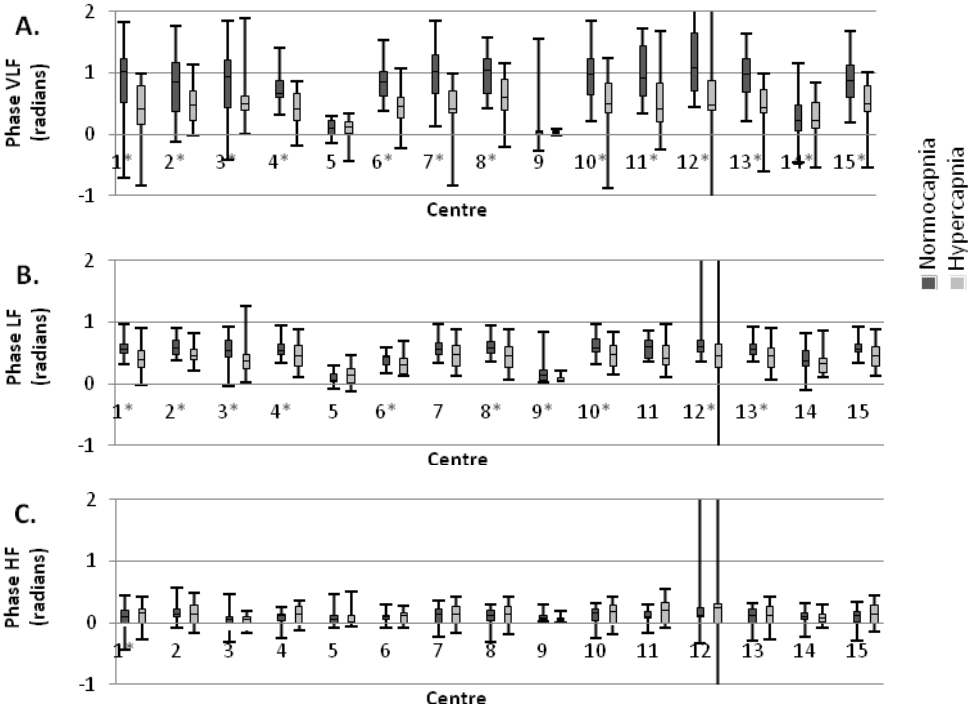

Figure 4. Phase results in radians (y-axis) for each centre (x-axis) showing the differences found between normocapnia (dark boxes) and hypercapnia (light boxes). The spread between the results is represented using multiple box plots. On each box, the central mark is the median value, the edges of the box are the 25th and 75th percentiles and the whiskers extend to the most extreme data points. Asterisks (*) indicate that the centre found a significant difference between normocapnia and hypercapnia ($p < 0.05$). The graphs show the phase results for the VLF (A), LF (B) and HF (C).

Differences between centre outcomes

Table 3, 4 and 5 (Supplement) show the numerical values (median, 1st/3rd quartile, min, and max) for the results found for the 50 healthy subjects per centre. The relation between the data generated by the 15 centres for the 50 healthy subjects is presented in Figure 3. For all frequency bands, a cluster is formed by the following 9 centres: 1, 2, 4, 7, 8, 10, 11, 12 and 13.

Hypercapnia

Figure 4 and 5 show results for phase and gain in VLF (top), LF (middle), and HF (bottom) for each centre (represented on the x-axis) obtained in the 20 subjects under normal resting conditions (normocapnia) and for these same 20 subjects during 5% CO_2 breathing (hypercapnia), using multiple box plots.

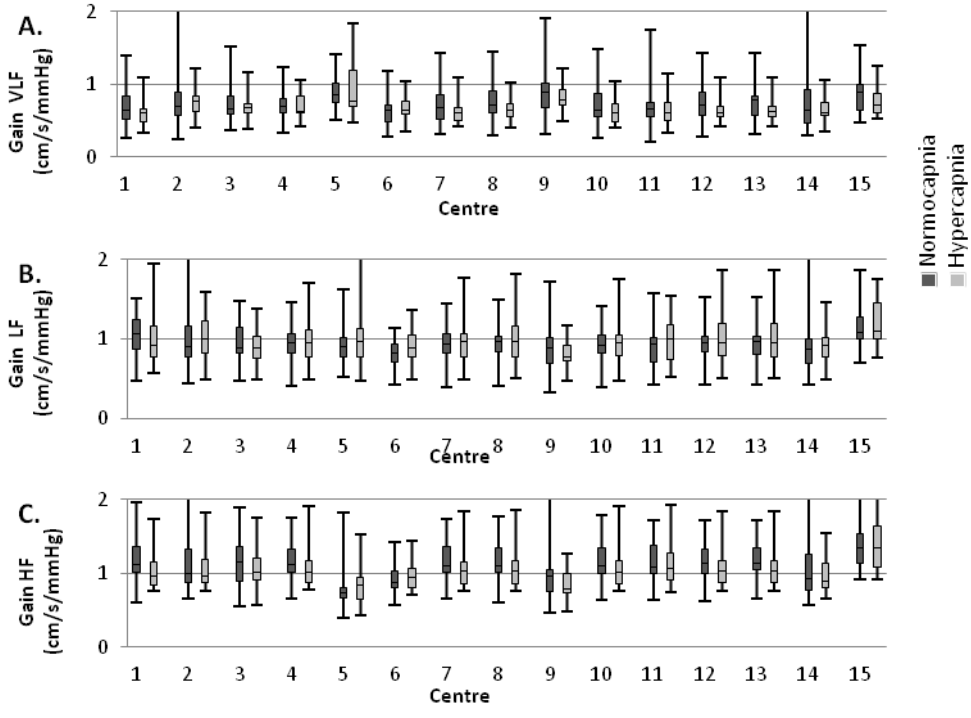

Figure 5. Gain results in cm/s/mmHg (y-axis) for each centre (x-axis) showing the differences found between normocapnia (dark boxes) and hypercapnia (light boxes). The spread between the results is represented using multiple box plots. On each box, the central mark is the median value, the edges of the box are the 25th and 75th percentiles and the whiskers extend to the most extreme data points. Asterisks (*) indicate that the centre found a significant difference between normocapnia and hypercapnia ($p < 0.05$). The graphs show the phase results for the VLF (A), LF (B) and HF (C).

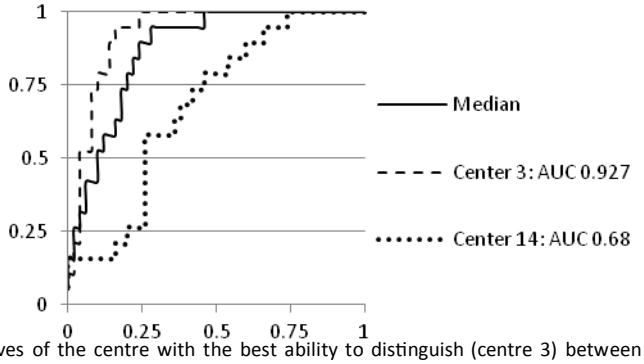

Figure 6. ROC curves of the centre with the best ability to distinguish (centre 3) between normocapnia and hypercapnia and the centre which is least able to distinguish (centre 14). ROC curves are derived using logistic regression. Logistic regression was performed using the state of CA (normal = baseline, impaired = hypercapnia) as the outcome value and as predictor variables the phase VLF, phase LF, phase HF, gain VLF, gain LF, gain HF, coherence VLF, coherence LF and coherence HF.

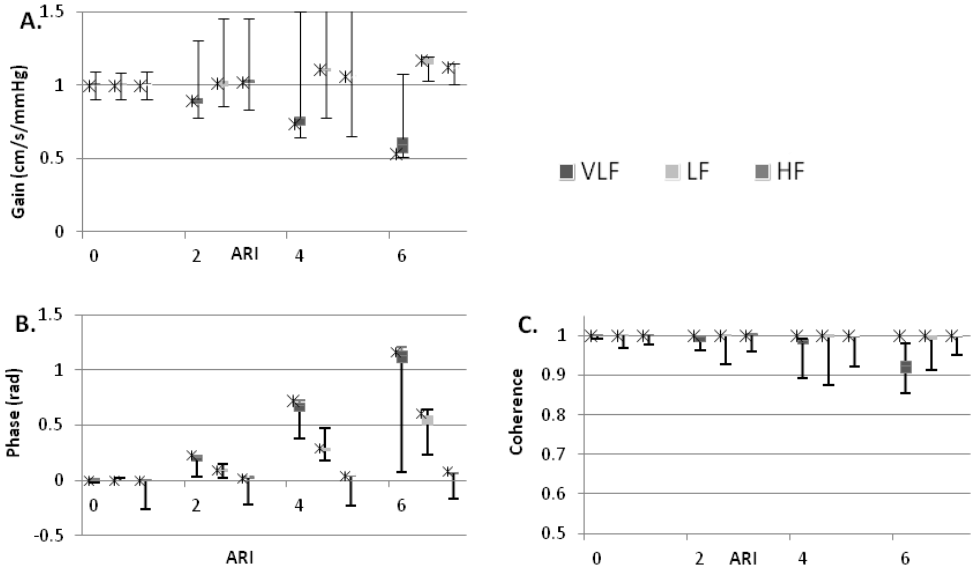

Figure 7. Gain (A), phase (B) and coherence (C) results (y-axis) for ARI 0, 2, 4 and 6. The spread between the centres is represented using multiple box plots. On each box, the central mark is the median value of the centres of the specific subject, the edges of the box are the 25th and 75th percentiles and the whiskers extend to the most extreme data points. Results are shown for the VLF, LF, and HF. Asterisks (*) indicate the values for transfer function phase and gain that were obtained by the software (written in Matlab by DS) that was used to generate the datasets.

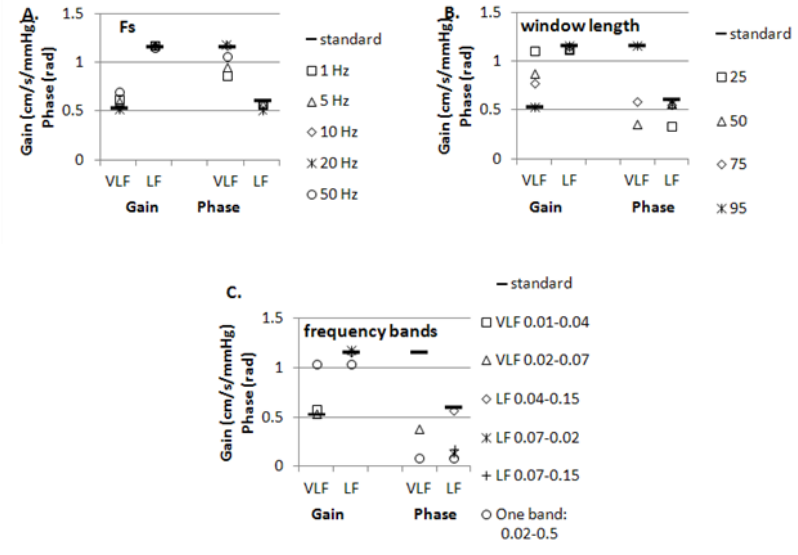

Figure 8. The transfer function results expressed in VLF gain, LF gain, VLF phase, and LF phase of ARI = 6 for three different parameter settings: sample frequency (1, 5, 10, 20 and 50 Hz) (A), window length (25, 50, 75 and 95 s) (B), and different frequency bands (C). Standard indicates the actual values of gain and phase of the 10 ARI models were used as criterion standard which were used as criterion standard value.

Logistic regression was performed using the two conditions of CA (normal = baseline, modified= hypercapnia) as outcome value, and as predictor variables the phase VLF, phase LF, phase HF, gain VLF, gain LF, gain HF, coherence VLF, coherence LF, and coherence HF.
Figure 6 shows the ROC that were generated for all centres, describing their performance in separating normal from modified. The graph shows ROC curves for the best (centre 3 AUC: 0.927), the worst (centre 14 AUC: 0.68), and the median of all centres.

Artificial datasets

Gain (top), phase (middle) and coherence (bottom) results (y-axis) for ARI 0, 2, 4 and 6 are shown in Figure 7. Results are shown as median, 1st and 3rd quartile, minimum and maximum values of the results of all centres for the VLF, LF and HF bands. The model for fully impaired autoregulation, ARI = 0, showed (as expected) a phase of nearly zero and a high gain (≈ 1 cm/s/mmHg). The model for intact CA, ARI = 6, shows a high phase (1.1 ± 0.4 rad) and a low gain (0.6 ±0.2 cm/s/mmHg) in the very low frequency.

Effect of different transfer function settings

The effect of three different parameter settings on transfer function gain and phase results for the ARI = 6 generated data are shown in Figure 8; sample frequency (1, 5, 10, 20 and 50 Hz), window length (25, 50, 75 and 95 s), and different frequency bands. The best results are found for a sample frequency of 10 Hz (the sample frequency used to generate the data). The farther the sample frequency deviates from 10 Hz, the more the results deviate from the criterion value. Decimating the data produces larger deviations than up-sampling of the data.

No differences were found in gain and phase estimates when different methods of interpolation were used (including spline versus linear interpolation), detrending versus no detrending (including removal of the mean, linear trend removal and third order polynomial detrending). Comparing the number of windows showed that using less than three windows resulted in less optimal results.

Also using window lengths smaller than 75 s resulted in larger deviations from the criterion value, particularly in the VLF. Using the Hamming or Hanning anti-leakage window did not result in different outcomes, but rectangular and Tukey window showed different results compared to the Hamming and Hanning windows. The rectangular window yields inferior results in LF gain and VLF phase and the Tukey window distorts the gain and the phase in the VLF. Varying the percentage of superposition, using percentages of 25 %, 50 % and 75 %, did not affect the outcomes. The use of different frequency bands affected outcomes for phase in VLF and in LF. Using a frequency band of 0.02 - 0.5 Hz led to the strongest deviation from the criterion value.

DISCUSSION

Strong diversity exists in the signal processing methods for TFA used by the international research community in quantifying CA. The objective of this multi-centre study was to evaluate how this diversity in methods affects transfer function outcomes for CA. An important underlying question was whether these between-centre differences in how to perform TFA may contribute to the large variability in TFA outcomes in the literature. This objective was achieved by asking different centres that use different methodological approaches for TFA, to analyse the same dataset. Our main finding is that TFA analysis — of the same dataset — by these centres led to a large variability in metric outcomes. This large variability could in part be explained by the use of different parameter settings for TFA by

these centres, however part of the variability remains unexplained. This finding of large between-centre variability is in agreement with the results of a systematic review of published TFA studies [2]. Our study adds to this review by directly comparing multiple centres that analyse the same set of data. Furthermore, we systematically evaluated how different choices for the signal processing methods will affect outcomes.

Non-invasive monitoring of CA is important, since disorders of CA may lead to adverse clinical outcomes such as cerebral hypoperfusion [10]. Currently, no gold standard test for the assessment of dynamic CA exists. TFA is the most widely used and reported method for non-invasive quantification of CA. However, no standardised form for the implementation of the transfer function exists, resulting in high diversity among studies [2]. Our study showed that the inter-centre variability is quite high, particularly for a procedure that is deemed rather straightforward and places restrictions on the choice of implementation parameters. Even though instructions were given to the centres that should have reduced variability, the observed high spread in CA outcomes was still due to diversity in TFA signal processing choices. Parameter settings that differed between the centres were: sampling frequency, type of interpolation, number of windows, window length, type of anti-leakage window, frequency resolution, type of spectral smoothing and phase unwrapping. Based on our analyses discussed below, we can conclude that these differences in settings are in part responsible for the high diversity in results from the different centres. Not all variance could however be fully explained by these settings. Nevertheless, despite the large variance, most centres showed good ability to distinguish between normal CA and modified CA (using hypercapnia). Even the poorest performing centre had a ROC AUC higher than 0.75.

Effect of different parameter settings on TFA outcome

To further investigate how different settings may affect TFA analysis, in a sub-study we used artificially generated datasets to systematically evaluate how different parameter settings affect transfer function outcome. These analyses were performed in a single centre. We did this by running repeated analyses of these artificial datasets, using analysis software written in Matlab® (by author DMS), each time changing a single parameter setting. These analyses showed that particularly the sampling frequency, number of windows, window length, type of anti-leakage window, and the definition of frequency bands influenced the results. Regarding the sampling frequency, we found that both decimating and up-sampling the data altered the results. A reduction of sample frequency resulted in a VLF phase decrease for the artificial data. When we compared the clustered centre data (1, 2, 4, 7, 8, 10, 11, 12 and 13) this VLF

phase decrease was confirmed by the results of centre 4 that used a sample frequency of 1 Hz. Also the centres 3, 9 and 14 used low sample frequencies and showed lower VLF phase. In the present study a sampling frequency of 10 Hz resulted in the best results. However, this could be due to the fact that the artificial datasets were created at 10 Hz. Therefore, a different sampling frequency may be optimal for biological signals of BP and CBFV; this awaits further investigation. Lowering the number of windows or the length of the window mainly affected the VLF outcomes, but this effect of increased VLF gain and decreased VLF phase with decreasing window length could not be confirmed by comparing the results in healthy subjects. However, since the pre-specified settings instructed the centres to use a window length of 90-100 seconds little between-centre variation in window length was present. Concerning the type of anti-leakage window, we found that the Hanning and Hamming window did not differ in outcome values. However, the use of a rectangular or Tukey window yielded inferior results. This can be explained by the fact that the rectangular window has excellent spectral resolution characteristics for sinusoids of comparable strength, but it is a poor choice for sinusoids of disparate amplitudes [6] and the Tukey window, also known as the tapered cosine window, has also relatively poor leakage protection. It should be pointed out that the analysis of synthetic data can only provide partial guidance into the optimal choices for estimator settings. For example, in the synthetic data, the input-output relationship was purely linear, and there was no noise present in the data. Thus the benefits of detrending or increased window-length present in signals recorded from human volunteers cannot be observed.

Implications

These results imply that standardisation of the parameter settings used to calculate the transfer function could substantially lower the inter-centre variability in transfer function outcomes. However, some methodological issues need to be considered. TFA is a linear method, while, in general, CA is considered to be a non-linear phenomenon. Therefore it is possible that other methods, i.e. non-linear approaches, may provide better estimates of CA . Although it is likely that variations in parameter settings may also influence metric outputs as observed here for TFA. We suggest that resolving the lack of methodological standardisation must take priority when new methods are proposed and implemented. Future studies should implement the approach taken in this study, with multiple centres analysing a central database, for a prospective study comparing different methods to analyse CA, to investigate whether non-linear methods are superior to TFA, while at the same time optimising standardisation of such methods. Another methodological issue is whether it is accurate to

assume that TFA wholly reflects dynamic CA. Although the relationship between cerebral perfusion pressure and flow are influenced by dynamic CA, it is important to recognise that other physiological factors such as baseline BP, PCO_2 [11, 12], brain metabolic activity, and sympathetic tone are also influential. Because TFA considers only the BP (input) and CBF (output) relationship, the approach is clearly a simplification of a highly complex physiological system. In this context, the contribution of PCO_2 as an additional input has been investigated by for example in [12, 13]. However, the exclusion of this (and other) inputs does not mean that the transfer function analysis has no clinical utility. Indeed, the medical literature is replete with examples of simplified measures that have been successfully applied as metrics for risk stratification and monitoring. A good example is the use of gait speed (measuring the time taken to walk 4 m) as a measure of the highly complex concept frailty, and even as a prediction of survival [14].

In conclusion, this study shows that there is an urgent need for detailed standardisation of the signal processing methods used for TFA. Without such standardisation, additional uncertainty is added to any comparison between studies of autoregulation carried out at different centres.

SUPPLEMENTARY APPENDIX

Table 1. Overview of the normal routine settings for transfer function analysis of each centre . NR is not reported.

Centre	1	2	3	4	5	6
Input data	beat to beat	beat to beat	beat to beat	beat to beat	raw wave-form	beat to beat
Sampling frequency (Hz)	4	5	2	1	1000	1
Type of interpolation in signal	spline	spline	none	none	none	none
Type of detrending	spline	spline	none	none	none	none
Number of windows	spline	spline	none	none	none	none
Window length in samples:	400	256	NR	64	128	depends on signal length
Type of anti-leakage window	Hanning	Hanning	NR	Hanning	512	Hamming
% of superposition	50	50	NR	50	50	50
Frequency resolution (Hz)	0.01	0.0195	0.005	0.003	0.007	0.004
Type of Spectral smoothing	NR	triangular	NR	none	none	none
Type of Phase unwrapping	none	arctan (mean (sin (phi))/ mean(cos (phi)))	none	Wrapped between -pi and pi	none	none
Cut-off level for coherence	0.5	0.2	none	none	NR	0.5

7	8	9	10	11	12	13	14	15
NR	beat to beat	beat to beat	beat to beat	raw wave-form	NR	raw wave-form	raw wave-form	beat to beat
NR	5	2	100	500	NR	50	50	5
NR	none	linear	linear	linear	NR	NR	polyphase implemen-tation, anti-aliasing (lowpass) FIR filter	linear
NR	none	polynomial	3rd order polynomial	NR	NR	2nd order	linear	none
NR	4 to 10	depends on signal length	5	NR	NR	NR	depends on signal length	4
NR	512	128	128	NR	NR	5000	depends on signal length	256
NR	Hanning	Hamming	Hanning	Hanning	NR	rectangle	Hanning	Hanning
NR	40	50	50	NR	NR	50	50	50
NR	0.098	NR	0.016	0.02	NR	NR	5	0.02
NR	triangular	none	none	triangular	NR	none	none	triangular
NR	remove negative values	remove negative values	none	NR	NR	none	none	none
NR	0.5	none	none	0.5	NR	0.5	none	none

Table 2. Overview of the used settings for transfer function analysis by the centres for this study. NR is not reported.

Centre	1	2	3	4	5	6
Input data	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat
Sampling frequency (Hz)	4	10	2	1	NR	10
Type of interpolation in signal	none	none	none	NR	none	none
Type of detrending	none	none	none	none	none	none
Number of windows	5	5	0	5	5	8
Window length in samples:	400	512	NR	NR	256	NR
Type of anti-leakage window	Hanning	Hanning	NR	Hanning	Hanning	Hamming
% of superposition	50	50	NR	50	50	50
Frequency resolution (Hz)	0.01	0.02	0.005	none	NR	NR
Type of Spectral smoothing	none	none	NR	NR	NR	none
Type of Phase unwrapping	none	arctan (mean (sin (phi))/ mean(cos (phi)))	none	NR	NR	none
Cut-off level for coherence	none	none	none	none	none	none

7	8	9	10	11	12	13	14	15
beat to beat	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat	beat to beat
10	5	2	10	10	10	NR	1	1
none	none	linear	linear	linear	none	NR	none	none
none	none	none	none	none	none	none	none	none
5	5	NR	5	5	5	5	5	5
1000	512	NR	1024	NR	1000	1000	90	100
Hanning	Hanning	Hanning	Hanning	Hanning	Hanning	Hanning	Hanning	hamming
50	50	50	50	50	50	50	50	50
0.01	none	none	none	NR	0.01	none	0.02	0.003
none	none	none	none	triangular	none	none	none	none
none	none	none	none	NR	none	none	none	none
none	none	none	none	none	none	none	none	none

Table 3. The numerical values for the phase (median, $1^{st}/3^{rd}$ quartile, min, and max) for the results found for the 50 healthy subjects per centre.

Phase (rad)															
Centre	Very low frequency					Low frequency					High frequency				
	median	1st quartile	3rd quartile	min	max	median	1st quartile	3rd quartile	min	max	media	1st quartile	3rd quartile	min	max
1	0.7	0.4	1.1	-0.7	1.83	0.6	0.5	0.7	-0.2	0.99	0.13	-0	0.3	-0.4	1
2	0.9	0.4	1.2	-0.7	1.8	0.6	0.5	0.8	0.31	1.28	0.2	0.08	0.3	-0.2	1
3	0.7	0.3	1	-0.4	1.86	0.5	0.4	0.7	-0.2	1.41	-0.1	-0.2	0	-0.4	1
4	0.6	0.4	0.9	-0.3	1.4	0.6	0.5	0.8	0.17	1.31	0.15	0.05	0.2	-0.3	1
5	0.2	0	0.2	-0.7	0.41	0.2	0.1	0.4	-0.9	0.83	0.17	0.01	1	-1.3	1.59
6	0.7	0.5	1	-0.2	1.58	0.4	0.3	0.5	-0.1	0.91	0.13	0.06	0.2	-0.1	1
7	0.8	0.5	1.1	-0.6	1.84	0.6	0.5	0.9	-0	1.42	0.18	0.09	0.3	-0.4	1
8	0.8	0.5	1.2	-0.2	1.58	0.6	0.5	0.8	0.16	1.36	0.19	0.07	0.3	-0.4	1
9	0	0	0.1	-0.3	1.55	0.1	0.1	0.2	-0	1.07	0.06	0.03	0.1	-0.1	1
10	0.7	0.3	1.1	-0.7	1.84	0.6	0.5	0.8	0.11	1.41	0.2	0.08	0.3	-0.3	1
11	0.7	0.4	1.1	-0.8	1.73	0.6	0.4	0.7	-0.2	1.19	0.15	0.06	0.3	-0.3	1
12	0.9	0.6	1.3	-0.1	7.69	0.6	0.5	1	0.14	7.26	0.22	0.08	0.4	-0.7	8.75
13	0.8	0.5	1.1	-0.4	1.63	0.6	0.5	0.8	-0.4	1.31	0.17	0.05	0.3	-0.3	1
14	0.4	0.2	0.7	-0.5	1.62	0.4	0.2	0.6	-0.1	1.11	0.11	0.04	0.2	-0.2	1
15	0.7	0.5	1	-0.3	1.67	0.6	0.5	0.8	0.01	1.25	0.19	0.07	0.3	-0.3	1

Table 4. The numerical values for the gain (median, 1st/3rd quartile, min, and max) for the results found for the 50 healthy subjects per centre.

Gain (cm/s/mmHg)															
Centre	Very low frequency					Low frequency					High frequency				
	median	1st quartile	3rd quartile	min	max	median	1st quartile	3rd quartile	min	max	media	1st quartile	3rd quartile	min	max
1	0.67	0.52	0.93	0.27	3.3	0.96	0.83	1.3	0.31	4.2	1.1	0.89	1.4	0.54	3.1
2	0.69	0.53	0.97	0.25	3.1	0.99	0.73	1.2	0.29	4.3	1.1	0.87	1.4	0.48	3.9
3	0.64	0.54	0.91	0.28	3.4	0.9	0.75	1.2	0.45	4.1	1.11	0.89	1.4	0.51	3.9
4	0.7	0.55	0.91	0.29	3.4	0.95	0.75	1.1	0.33	4.3	1.1	0.88	1.4	0.39	3.3
5	0.76	0.63	0.96	0.22	2.6	0.76	0.57	0.95	0.19	2.62	0.51	0.21	0.72	-0.14	1.8
6	0.68	0.52	0.87	0.27	3.5	0.86	0.71	1.02	0.31	3.4	0.91	0.79	1.2	0.41	2.7
7	0.71	0.52	0.97	0.27	3.25	0.93	0.75	1.1	0.35	4.3	1.1	0.9	1.4	0.5	3.3
8	0.73	0.56	0.93	0.28	3.84	0.96	0.76	1.2	0.35	4.3	1.1	0.91	1.37	0.5	3.3
9	0.88	0.64	1.0	0.24	2.04	0.86	0.67	1.0	0.27	2.2	0.94	0.68	1.1	0.37	2.1
10	0.7	0.52	0.92	0.25	3.2	0.92	0.75	1.1	0.33	4.3	1.1	0.9	1.4	0.5	3.3
11	0.71	0.54	1.0	0.2	3.7	0.93	0.65	1.1	0.3	4.5	1.05	0.81	1.4	0.43	3.9
12	0.72	0.57	0.91	0.27	3.4	0.93	0.77	1.2	0.34	4.3	1.1	0.89	1.4	0.5	3.3
13	0.76	0.57	0.92	0.31	3.4	0.93	0.77	1.2	0.34	4.3	1.1	0.89	1.4	0.5	3.3
14	0.7	0.51	0.97	0.29	3.61	0.86	0.68	1.06	0.26	3.7	0.92	0.79	1.3	0.42	2.8
15	0.89	0.68	1.2	0.38	2.1	1.1	0.97	1.3	0.64	2.31	1.3	1.1	1.7	0.67	2.8

Table 5. The numerical values for the coherence (median, 1st/3rd quartile, min, and max) for the results found for the 50 healthy subjects per centre.

Coherence															
Centre	Very low frequency					Low frequency					High frequency				
	median	1st quartile	3rd quartile	min	max	median	1st quartile	3rd quartile	min	max	media	1st quartile	3rd quartile	min	max
1	0.43	0.34	0.61	0.22	0.79	0.76	0.63	0.83	0.3	0.91	0.67	0.54	0.74	0.23	1
2	0.5	0.42	0.62	0.22	0.83	0.74	0.6	0.83	0.18	0.93	0.65	0.53	0.72	0.14	1
3	0.68	0.52	0.78	0.32	0.97	0.88	0.79	0.93	0.48	0.99	0.82	0.74	0.91	0.51	1
4	0.52	0.47	0.66	0.28	0.83	0.74	0.62	0.82	0.28	0.91	0.65	0.56	0.74	0.3	1
5	0.53	0.33	0.67	0.05	0.97	0.59	0.42	0.74	0.07	0.97	0.4	0.26	0.59	0.09	0.96
6	0.47	0.37	0.58	0.23	0.79	0.73	0.63	0.8	0.38	0.89	0.72	0.64	0.79	0.33	1
7	0.45	0.35	0.63	0.22	0.79	0.75	0.63	0.82	0.3	0.92	0.69	0.57	0.74	0.25	1
8	0.53	0.41	0.62	0.3	0.84	0.75	0.59	0.83	0.23	0.93	0.68	0.56	0.75	0.23	1
9	0.92	0.85	0.95	0.33	0.99	0.85	0.8	0.9	0.6	0.97	0.9	0.84	0.93	0.67	1
10	0.45	0.34	0.64	0.2	0.78	0.76	0.62	0.81	0.3	0.92	0.68	0.58	0.74	0.24	1
11	0.47	0.34	0.65	0.17	0.85	0.75	0.58	0.82	0.21	0.96	0.64	0.52	0.76	0.17	1
12	0.5	0.38	0.64	0.29	0.77	0.75	0.6	0.82	0.23	0.93	0.67	0.54	0.74	0.23	1
13	0.48	0.39	0.64	0.29	0.77	0.75	0.6	0.82	0.23	0.93	0.67	0.55	0.74	0.23	1
14	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
15	0.49	0.4	0.63	0.3	0.79	0.74	0.62	0.82	0.27	0.92	0.68	0.57	0.73	0.25	1

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Chapter 4.

Preliminary guideline for transfer function analysis

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ABSTRACT

The brain is highly dependent on a continuous supply of (oxygenated) blood. Cerebral autoregulation (CA) is a key mechanism to protect the brain against excessive fluctuations in blood pressure (BP) to maintain adequate cerebral blood flow. The ability to measure and monitor CA in a patient would provide clinically useful information and would possibly permit a more individualized physiologically based therapy aimed at reducing the risk of secondary brain injury.

A widely used technique to quantify CA in a non-invasive way is analysing the relationship between spontaneous BP and cerebral blood flow velocity (CBFV) using transfer function analysis (TFA). Despite the frequent use of TFA, however, settings and qualifying terms for the interpretation of outcome metrics, for example criteria for “impaired CA”, have never been strictly defined, leading to limitations of comparison between existing literature, poor standardization and postponement of potential clinical benefits.

The purpose of the present guideline is to establish a first consensus document on CA quantification using TFA with the use of BP and CBFV signals (obtained with transcranial Doppler ultrasonography (TCD)), to improve reproducibility and implementation of study results.

The development of these guidelines was initiated by (but not confined to) The Cerebral Autoregulation Research Network (CARNet - www.car-net.org). This document reflects what is currently considered best practice in awake and cooperating patients with TCD monitoring. The proposed settings are not intended to necessarily indicate what is ‘best’ (by whatever criterion) and we emphasize that the discussion is ongoing, probably leading to future revisions in the light of new evidence. As a first step towards implementation, we encourage researchers to compare their practice to the settings as they are recommended in this consensus paper.

INTRODUCTION

Perfusion of the human brain is more or less maintained by a set of control systems including chemo- and autoregulation, neurovascular coupling and probably a direct autonomic neurovascular influence. Myogenic mechanisms are traditionally represented by the brain's capacity to autoregulate its own blood flow and cerebral autoregulation (CA) is accordingly defined as the intrinsic ability of the brain to maintain cerebral perfusion in the presence of blood pressure (BP) changes. As the brain is highly dependent on a continuous supply of (oxygenated) blood, reduced effectiveness of CA renders the brain more sensitive to both hypo- and hyperperfusion. In a variety of medical conditions, such as serious hypertension, diabetes, dementia, stroke, head trauma, subarachnoid haemorrhage as well as during surgical procedures [1-5], defect CA may play an important role in the pathogenesis of brain damage. Therefore, the ability to measure and monitor CA in a patient would provide clinically useful information and would possibly permit a more individualized physiological based therapy aimed at reducing the risk of secondary brain injury.

The cerebral autoregulatory mechanism was first proposed by Lassen et al. [6]. They proposed that CA works within a certain range of BP (≈ 60 to 150 mmHg). Outside this, so-called autoregulatory range, vasomotor adjustments are exhausted and cerebral blood flow becomes pressure-passive and subjected to changes in BP. This view on CA is called 'static autoregulation'. Over the last two decades, techniques with high temporal resolution (e.g. transcranial Doppler ultrasonography (TCD)) have shown that the relationship between BP and cerebral blood flow (CBF) is more dynamic, with short-term reactions. The dynamic relationship between BP and CBF has been shown to function as a high pass filter [7]. High frequency oscillations (> 0.20 Hz) in perfusion pressure are passed along unimpeded, while slower frequency oscillations (< 0.20 Hz) are dampened by the cerebral arterioles.

A large number of methods to assess the quality of CA have been proposed over the last 25 years. Traditional techniques to assess CA use changes in BP to challenge the cerebrovascular system. These BP changes can be induced using pharmacological means or with manoeuvres such as the Valsalva manoeuvre, squat-to-stand and/or sit to stand and the deflation of thigh cuffs [8-10]. However, these interventions are most of the time unsuitable in clinical cases, such as in the severely ill or in older or cognitively impaired persons, because of the relatively large BP change, the requirement of cooperation of patients and the uncomfortable nature of the interventions. Furthermore, other physiological subsystems (e.g. sympathetic activation with Valsalva's manoeuvre [11] and cortical activation with visual or acoustic stimuli [12]) or

parameters (e.g. pCO_2 with squat- and/or sit to stand manoeuvres [9]) might be affected, potentially confounding the results. Therefore, different research groups have adopted methods that use spontaneous (e.g. physiological) instead of induced slow BP fluctuations to challenge CA.

Because of the recognition of the potential clinical importance of CA, numerous methods have been developed for non-invasive assessment of CA [13], including for example correlation coefficient analysis, the autoregulatory index [14], transfer function analysis (TFA) [15], nonlinear analyses using Laguerre expansions of Volterra kernels or Principal Dynamic Modes [16], autoregressive [17], as well as multi-modal pressure-flow analysis [18].

With the existence of this multitude of methods, the choice of which method to employ to quantify CA remained a matter of personal choice. No single method has been universally accepted as the gold standard.

Of all available methods, TFA is the most frequently used in the literature to quantify CA using spontaneous fluctuations in BP and CBF [19]. Transfer function analysis is based on analysis of frequency components of oscillations in BP and the resultant degree to which these oscillations are reflected in cerebral blood flow velocity (CBFV) [7]. One of the advantages to this method is that it only takes a baseline measurement without the need for any pharmacological or physiological manipulation of BP. Transfer function analysis starts with dividing the cross spectra between blood pressure and cerebral blood flow velocity by the auto spectra of the blood pressure. With the transfer function the associated relative power (gain), timing (phase) and the linear association (coherence) can be described. Evaluation of CA by TFA is based on the concept that CA minimizes the effect of dynamic BP fluctuations on CBFV, which is reflected by reduced low-frequency gain and phase-lead of CBFV over BP. Without CA, CBFV would passively follow BP and TFA would show constant gain and zero phase across the low-frequency band. To estimate the cross and power spectra several parameter settings need to be chosen, such as sample frequency, window length, overlap percentage and filtering. Despite the frequent use of TFA, however, settings and qualifying terms for the interpretation of outcome metrics, for example criteria for “impaired CA”, have never been strictly defined, leading to limitations of comparison between existing literature, poor standardization and postponement of potential clinical benefits.

The purpose of the present guideline is to establish a first consensus document on CA quantification using TFA with the use of BP and TCD CBFV signals. The guidelines focus on TCD

CBFV signals, as TCD CBFV is currently the most used non-invasive surrogate for CBF. It must be noted that the use of another surrogate, such as oxygenation index obtained with near-infrared spectroscopy [20], may require different TFA settings.

The development of these guidelines was initiated by (but not confined to) The Cerebral Autoregulation Research Network (CARNet - www.car-net.org). This document reflects what is currently considered best practice in awake and cooperating patients with TCD monitoring, although the discussion is ongoing, probably leading to future revisions in the light of new evidence.

It should be noted that the choice for TFA in this document should not be seen as a statement that TFA is considered the best available method to quantify CA.

METHOD

Related to the subject of the present paper, a systematic review of the TFA literature has been performed [21]. That paper (which includes details on search strategy and study inclusion) has identified a large diversity in the signal processing methods, experimental conditions and research protocols that have been used for TFA in previous publications, and the paper provides an overview of this heterogeneity in methods [21]. Following this review, a multi-centre study wherein a single, uniform database with healthy patients with BP and TCD recordings was analysed by different research centres, each using their own TFA settings (filters, frequencies, etc), which was initiated and carried out as part of CARNet. The results of the systematic review and multicenter study were discussed during the Second CARNet International Conference in Nijmegen, September 2012. To derive the guidelines, we then combined the results of the systematic review and the multi-centre study with other available evidence-based scientific literature and with expert opinions obtained from within CARNet. Next, the initial proposal for consensus guidelines was discussed during the 3rd CARNet International conference in Porto, May 2013. During this conference, the arguments in support of the consensus proposal were presented followed by an open discussion with all the participants, and the manuscript was amended accordingly. The CARNet consensus group on TFA listed at the end of the manuscript includes all those who contributed to the consensus process and the preparation of this consensus paper.

The various topics related to the use of TFA for CA are divided into 3 different subjects: experimental procedure, TFA methodology and documentation of TFA results.

GUIDELINE PROPOSAL

EXPERIMENTAL PROCEDURES

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Although the main purpose of the consensus document is to standardize TFA, consistent and reliable results cannot be obtained unless due care is given to data acquisition and measurement protocols. These include environmental conditions, body position, measurement technique and control of physiological covariates that can influence CA.

Consensus statement 1

Subjects should refrain from the ingestion of a heavy meal for at least 4 hours before examination. Exercise and caffeine and alcohol ingestion should also be avoided for a minimum of 12-hours. Supplement and various medications may also affect TFA and should be accounted for depending on the experimental question. If the resting state of CA is studied, visual or acoustic stimulation should be kept to a minimum (no disturbances by people entering or leaving the room). Sensory stimuli may however be applied in a controlled way if CA analysis is focusing on neurovascular coupling. Adequate explanation of the procedure to the subject will help to reduce fear and anxiety, especially in anxious individuals. End-tidal gases should be used to confirm the absence of hyperventilation, because of known diurnal variation in CA [22]. It is recommended that tests be conducted at a similar time of day for repeat assessments and, for between-group studies, assessment times should be standardized.

Consensus statement 2

BP can be measured invasively through an arterial line or non-invasively, through arterial volume clamping applied to a finger or via tonometry at the radial artery [23]. For the latter two methods, the finger should be supported at heart level or a height correction needs to be used. It is recommended that values of systolic and diastolic BP are obtained by standard sphygmomanometry prior to each recording, to ascertain reliable BP values.

TCD CBFV can be measured non-invasively in the middle cerebral arteries (MCA) using transcranial Doppler ultrasonography, using ultrasound probes placed over the temporal window, or from other intracranial arteries such as the anterior cerebral artery (ACA) or posterior cerebral artery (PCA) [24]. The insonated artery should be identified according to its

signal depth, velocity and wave characteristics [24].

Consensus statement 3

Recordings of spontaneous fluctuations of BP and CBFV for TFA should last a minimum of 5 minutes, assuming stationary physiological conditions and uninterrupted good quality data, to ensure that recordings can yield robust estimates of transfer function parameters and to improve frequency resolution as detailed in the following statements. For a resting state or baseline level of CA, BP and CBFV should be measured with uncrossed legs after a 15 min period of rest. All body positions should be clearly reported (e.g. supine with head 30° elevation).

Consensus statement 4

Given the strong effects of carbon dioxide pressure (PCO_2) on dynamic CA and BP, it is important to incorporate its measurement simultaneously with BP and CBFV. This is often accomplished by recording the trend of end-tidal CO_2 signal with infra-red capnography or mass spectroscopy. Any significant fluctuations in PCO_2 (> 1 mmHg) during the recording should be reported and taken into consideration when interpreting results from TFA [25]. If intracranial pressure monitoring is performed pressure levels at which dynamic CA analysis is performed will have to be given as well. This is because intracranial hypertension may impair CA [26].

TFA METHODOLOGY

TFA is usually computed by way of the fast Fourier transform (FFT) to obtain estimates of auto- and cross-spectra. Spectral estimates derived from a single data window show considerable scatter with coefficients of variation (CoV = standard deviation / mean) approximately equal to one [27]. To improve precision of spectral estimations, averaging or smoothing is required, often using Welch's method [28] which breaks the total data length into separate segments or windows to obtain averaged auto- and cross-spectra. In addition to signal segmentation, spectra may also be smoothed using moving-average filters in the frequency domain. Due to the phenomenon of spectral leakage [27], a tapered window must be applied to each segment of data in the time domain, before calculation of the FFT.

Selecting optimal parameter settings

104 The systematic review on TFA showed a marked diversity in the signal processing methods used for TFA [21]. Many different settings are used in the literature and variations are found in parameters such as type of data (raw or beat-averaged), sampling frequency, interpolation, detrending, normalization, filtering, length and type of window, and number and superposition of data segments when estimating power spectral densities [21]. Consensus recommendations for these parameters are provided below. Accurate reporting of TFA settings must be ensured, as it has been shown that small differences in settings may cause large variations in TFA outcomes, making results less comparable [19, 21].

Waveform vs. beat-to-beat data

The format of the BP and CBFV time signal used for TFA, i.e. the raw waveform of recorded signals or beat-to-beat data provides two alternatives used in the literature. No studies have been performed to compare TFA outcomes between the use of full waveform time signals or beat-to-beat data.

Consensus statement 5

Since most studies report using beat-to-beat data to analyse TFA [21], for the purpose of standardisation the use of beat-to-beat data is recommended for future research.

Sampling frequency

The literature also shows a wide range of sampling frequencies (e.g. 10 and 200 Hz [21]), the number of times per second the data, used as input for TFA, is sampled.

Consensus statement 6

For recording the continuous BP and CBFV signals, a minimum sampling frequency of 50 Hz is recommended, following anti-alias filtering. For mean values of BP and CBFV obtained from each cardiac cycle (beat-to-beat data) the minimum resampling frequency should be 1 Hz after interpolation (to get uniform time-axis). In order to avoid aliasing, beat-to-beat data should be interpolated (see below) first to 5 or 10 Hz (greater than the heart-rate). Following this, an anti-alias filter with a cut-off frequency below half the intended sampling rate (e.g. 0.4 times sampling rate) should be utilized, before resampling to the lower sampling rate.

Consensus statement 7

Signals should always be visually inspected prior to analysis (preferably considering both raw and beat-averaged signals simultaneously), to ensure that they are free of excessive noise and artefacts (for example signal artefacts induced by motion of the subject). Short periods of strong artefact (up to 3 beats) should be removed and replaced by linear interpolation. When excessive artefact persists for longer periods, the data segment should be excluded from analysis. Sporadic ectopic beats can be included in analysis [29, 30].

Interpolation

TFA supposes equidistant data points. Interpolation can be used to create equidistant time intervals (in case of beat-to-beat data), by constructing new data points within the range of a discrete set of known data points. Different types of interpolation, such as linear- and spline interpolation, are available, but it has been shown that this choice does not affect TFA outcomes considerably [19].

Consensus statement 8

To standardize inter-centre procedures, it is recommended that spline (3rd order polynomials) are used to interpolate the time-series of mean BP and mean CBFV calculated for each cardiac cycle.

Detrending

Detrending reduces the amount of low-frequency power by removing any linear or non-linear trends from the input data. It has been shown that the type of detrending used (including no detrending, removal of the mean, linear trend removal and third order polynomial detrending) does not affect TFA outcomes [19, 31]. However, this was tested in computer-generated (synthetic) data in which the input-output relationship was purely linear and no noise was present. This may explain why neither benefits nor disadvantages of detrending were observed, as may be seen for signals recorded from human volunteers. Further studies using synthesized data with added noise, including signal drifts, should be performed to confirm these observations.

Consensus statement 9

To standardize inter-centre procedures it is recommended that detrending is not used.

Units of measurement

106 The coherence function is dimensionless. Regarding the units of TFA estimate phase, the literature shows an almost equal divide between the use of radians and degrees and changing from one to the other is straightforward ($\alpha_{deg} = \alpha_{rad} / \pi$ x180) , where α_{deg} refers to the angle in degrees and α_{rad} to the angle in radians). However, when it comes to units of gain, the choice of which units to adopt is not so obvious. With measurements at rest, spontaneous fluctuations in CBFV and BP will normally be < 10% of their average value. For calculation of the FFT, it is normal practice to remove mean values (which should be reported as discussed later). This would reduce the inter-subject variability of the CBFV measure and therefore also of the gain. The existing options are to express both variables in absolute units or as relative values thus corresponding to $cm.s^{-1}.mmHg^{-1}$, $\%.mmHg^{-1}$, %/% or $cm.s^{-1}$/% [21]. Ideally, the units adopted for gain should be those that would maximize sensitivity and specificity for various conditions, but unfortunately studies of this question are lacking. Because of the unknown insonation angle and inter-subject differences that can occur in these angles comparison of absolute values of CBFV is limited. Furthermore, BP changes, of for example 10%, are physiologically very distinct for say a baseline mean BP of 90 or 150 mmHg. Therefore, expressing CBFV and BP as percentage changes with respect to their average value may be of preference. As a result, the choice would be between $cm.s^{-1}.mmHg^{-1}$ and $\%.\%^{-1}$.

Consensus statement 10

Estimates of gain obtained by TFA should be expressed in both absolute units ($cm.s^{-1}.mmHg^{-1}$) as well as %/%.

Filtering

Filtering can be used to delete frequencies that are of no interest, such as very high frequencies or extremely low frequencies. Meel-van den Abeelen et al. reported that in the literature only a minority of studies reported the use of any kind of filtering (11 %) [21]. The effect of filtering on TFA outcomes has not yet been investigated. However, it is expected that , filtering in frequency bins outside the frequency range used for analysis has no effect on the results.

Consensus statement 11

For the purpose of standardization, filtering is not recommended.

Anti-leakage window

Data segments need to be windowed to minimise a particular kind of distortion called spectral leakage [32]. A number of different shapes for anti-leakage windows, such as Hanning, Hamming and Tukey window [33], have been proposed and used. The multi-centre study, comparing the results of different centre methodologies for TFA, showed that there were no important differences in outcomes between using the Hamming or the Hanning anti-leakage window. In contrast, the use of a rectangular window (no anti-leakage) yielded inferior results in low frequency (0.07 - 0.15 Hz) gain and very low frequency (0.02 - 0.07 Hz) phase and the Tukey window distorted the gain and the phase in the very low frequency [19]. However, this was based on simulated noise-free signals and the impact on recorded signals has not been evaluated in the context of CA.

Consensus statement 12

Despite the lack of significant differences between the Hamming and Hanning anti-leakage windows, in the interest of standardization of results, the Hanning window is recommended [33].

Window length when using the Welch algorithm

The number of data points included in a window in combination with the sample frequency determines the window length in seconds, which will then define the frequency resolution of the transfer function estimates ($\Delta f = 1 / T$, where Δf is the frequency resolution in Hz and T the duration of the window, in seconds). It was shown in simulations that window lengths smaller than 75 seconds resulted in larger deviations from the expected values, particularly in the very low frequency range [19].

Consensus statement 13

To allow sufficient frequency resolution, it is recommended to use window segments with a length of > 75 seconds. When recordings longer than 5 minutes are available, it is preferable to increase the number of windows, rather than the length of individual windows when using the Welch algorithm.

Number and superposition of data segments

As described previously, the dominant technique in the literature for calculation of the
108 auto- and cross-spectra is the use of the FFT, combined with Welch's method [28] to improve its statistical reliability. This involves averaging spectral estimates from separate segments of data, which can be overlapped by variable degrees of superposition to maximize the degree of smoothing. Varying the percentage of superposition, using percentages of 25 %, 50 % and 75 %, has been shown not to affect the outcomes of TFA [19], with 50% being the most common degree of superposition reported in the literature.

Consensus statement 14

A superposition of 50 % is recommended for overlapping data segments in conjunction with Welch's method. Combining this recommendation with the minimum duration of the entire recording (minimum 5 min) and the window length (100 s) means that a minimum of 5 separate segments of data should be used with Welch's method. When recordings longer than 5 minutes are available, the window length should not be increased, but rather the number of windows should increase.

Phase wrap-around

Estimates of phase require the calculation of the tangent (tg^{-1}) of the ratio of the imaginary and real parts of the complex transfer function [27]. Unfortunately, the inverse tg^{-1} calculation cannot differentiate values of phase between π and 2 π radians from corresponding negative angles. This indetermination of phase estimates, usually referred to as 'wrap-around' can obviously lead to major distortions if negative values are mixed with positive ones when averaging phase for a group of subjects or across frequencies.

Consensus statement 15

The complete phase frequency response for each subject needs to be visually inspected to check for the occurrence of 'wrap-around'. When negative values of phase are detected for frequencies < 0.1 Hz, the particular recording should be removed [34].

Reporting TFA results

Next to accurate documentation of the settings used for TFA, it is important to report TFA outcomes in a standardized manner. In the earlier systematic review [5] it has been shown that TFA outcomes are presented in many different ways [21]. Overall, studies report the mean of the gain, phase and coherence over pre-defined frequency bands. Historically the most often used frequency bands for this purpose are 0.02 - 0.07 Hz for the VLF, 0.07 - 0.2 Hz for the LF and 0.2 - 0.3 Hz for the HF [35]. The upper limit for the VLF band was first defined by Zhang et al. [7], based on the observation that at ~ 0.07 Hz, the coherence crossed above 0.5, indicating 50 % shared variance between BP and DBFV at frequencies > 0.07 Hz. However, evidence about their sensitivity and specificity in detecting impairment of CA in different patient groups and/or physiological conditions is lacking.

Consensus statement 16

When reporting results of TFA, it is important to present the complete frequency dependence of coherence, gain and phase in the range 0.0 - 0.4 Hz, as mean and SD values at each frequency. Until further evidence is available, statistical analyses should be based on averaged values for the VLF (0.02 - 0.07 Hz), LF (0.07 - 0.2 Hz) and HF (0.2 - 0.3 Hz). In addition, mean values of BP and CBFV and their intra-recording variability should also be reported.

IMPLEMENTATION

Translating guidelines into daily clinical practice and research remains a challenge. The principal goal of the present guidelines is to improve reproducibility and implementation of study results. Table 1 shows an overview of the above mentioned guidelines for TFA to quantify CA.

As a first step towards implementation, we encourage researchers to compare their practice to the settings as they are recommended in this consensus paper. While some researchers may have strong arguments for not adopting the proposed settings, we strongly encourage all colleagues to present results with these settings (even if only as additional results), in order to facilitate comparison between studies and centres. Arguments against the use of the 'standard' setting should be provided in order to help shape future (changed) guidelines. The proposed settings are not intended to necessarily indicate what is 'best' (by whatever criterion) and we emphasize that currently the evidence in support of some of the proposed

Table 1. Overview of the guidelines for transfer function analysis to quantify cerebral autoregulation from spontaneous fluctuation in blood pressure and cerebral blood flow velocity.

Guidelines	
TFA parameter settings	
Input data	beat-to-beat data
Sample frequency	≥1 Hz
Interpolation	spline
Detrending	None
Normalization	None
Filtering	None
Anti-leakage window	Hanning
Window length	≥100 s
Superposition	50%
Reporting TFA results	Report mean of the gain, phase and coherence over the complete frequency range of 0.0 -0.5 Hz using a frequency plot with mean and standard deviation curves. Plus averaged values of the gain, phase and coherence for the VLF (0.02-0.07 Hz), LF (0.07 - 0.2 Hz) and HF (0.2-0.3 Hz).
Other outcome measures	mean blood pressure mean cerebral blood flow velocity mean end-tidal CO_2

settings is still weak, but standardization is considered preferable to a continued large variations between centres.

DISCUSSION

This paper provides guidelines for the use of TFA for the quantification of dynamic CA using the signals BP and TCD CBFV.

The urgent need for guidelines was demonstrated by the evidence that a strong diversity exists within the international community in the signal processing methods used for TFA. Besides a multi-centre study which investigated the effect of several parameter settings on TFA outcomes, only a few studies have been published on the effect of different TFA settings [34]. Therefore, many areas of uncertainty exist in which more studies are still needed. Further optimization of the guidelines will be made possible by future research, taking advantage of the work that has already been done.

When outlining the directions in which future research could be most useful, the following topics come to mind:

1. More studies to investigate the effect of different TFA settings, such as the choice of data, normalization, window length and filtering.

2. Application of an international standard protocol for the validation of the CBFV signal should be encouraged. Ensuring good signal quality will possibly also reduce the variability in TFA outcomes, making it easier to compare different studies.

3. Studies to determine the reference values and diagnostic usefulness in specific populations, such as children, elderly and patients with specific clinical conditions.

4. Determination of TFA standard decision criteria based on TFA outcomes.

5. More attention should be paid to the experimental or clinical protocol. Investigating the question whether ‘rest’ is the best reference condition which body posture (supine/seated) and evaluate the usefulness of physiological manoeuvres.

6. Similar studies for other analytical techniques, such as the time domain correlation

coefficient analysis [26], non linear multi modal flow analysis [18] and dynamic non linear analysis using Laguerre expansions of Volterra kernels or Principal Dynamic Modes [14-15] and a comparison between methods with regard to their ability to identify impaired CA.

7. Understanding that CA is but one of the mechanisms involved in the control of cerebral perfusion. We need to know how it interacts with and is related to other mechanisms for CBF regulation, such as the arterial baroreflex.

Finally, we emphasize that these guidelines are not intended to endorse TFA as the 'best' method for the quantification of CA. One of the key limitations of TFA is that it assumes a linear relationship between BP and CBFV. In general, however, CA is considered to be a non-linear phenomenon. Other methods, i.e. non-linear approaches, may provide more reliable or sensitive estimates of CA, but in the continued absence of a gold standard for dynamic CA assessment, clear criteria and multi-centre trials on a wide range of patients would be required to allow robust conclusions. In the mean time, these guidelines show a recommended way to perform TFA, thereby providing a solid standard for comparison between studies, including those seeking to establish better CA analysis methods.

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Chapter 5.

Convergent cross mapping: a new non-linear method for the quantification of cerebral autoregulation

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ABSTRACT

Background

Cerebral autoregulation (CA) is the physiological mechanism that keeps the cerebral blood flow (CBF) relatively constant despite changes in blood pressure (BP). Currently, transfer function analysis (TFA) is widely used to assess CA non-invasively. TFA is based on the assumption that CA is a linear process, however, in reality CA has shown to be a non-linear process. This study explores the usability of convergent cross mapping (CCM) as a non-linear analysis technique to assess CA.

Material and Methods

CCM determines causality between variables by investigating if historical values of a time-series X(t) can be used to predict the states of a time-series Y(t). The Pearson correlation is determined between the measured Y(t) and the predicted Y(t) and increases with increasing time-series length to converge to a plateau value. When used for CA, normal and impaired CA should be distinguishable by a different plateau value. With impaired CA, BP will have a stronger influence on CBF, and therefore the CBF signal will contain more information on BP. As a result, the correlation converges to a higher plateau value compared to normal CA. The CCM method was validated by comparing normal CA (normocapnia: breathing 0 - 2 % CO_2) with a model of impaired CA (hypercapnia: breathing 6 - 7 % CO_2).

Results

CCM correlation was higher ($p = 0.01$) during hypercapnia (0.65 ± 0.16) compared to normocapnia (0.51 ± 0.18).

Conclusion

CCM is a promising technique for non-linear cerebral autoregulation estimation.

INTRODUCTION

The high metabolic demand of the brain requires an adequate cerebral blood flow (CBF). However, changes in blood pressure (BP) or intracranial pressure may influence CBF. To keep CBF relatively constant and to return CBF to baseline after a fast change in BP, adaption of the cerebrovascular resistance (CVR) occurs. This process is called cerebral autoregulation (CA) [3]. When CA is disturbed, the brain may become excessively sensitive to fluctuations in BP, causing hypo- and/or hyperperfusion. Hypo- and hyperperfusion can lead to ischemia or haemorrhages, respectively [4]. CA failure has been associated with increased morbidity and mortality [5]. Therefore, the ability of accurately quantifying the quality of CA may be of great importance in clinical practice.

CA can be determined as static CA or dynamic CA. With static CA, the response of the CBF to changes in BP is studied in a semi-steady state, i.e. a measurement of CBF is obtained first at a constant baseline BP and constant CBF, followed by another measurement that is taken after the autoregulatory response to a manipulation of BP has been completed [6]. However, static CA represents the overall effect of the autoregulatory action, but does not address the time in which this is achieved.

The use of Transcranial Doppler (TCD) ultrasound combined with servo-controlled finger photoplethysmography makes it possible to measure the process of CA itself, the dynamic CA [4, 7]. Ideally, clinical monitoring of cerebral autoregulation should be non-invasive, continuous, bedside and precise. Because static cerebral autoregulation measurements only provide steady-state point measurements and therefore is not a continuous measurement, the dynamic approach is preferable.

Despite the importance of measuring dynamic CA, there is no consensus about the best way to analyse dynamic CA [8]. Currently, the most frequently used method in literature is transfer function analysis (TFA) [8]. However, this method is based on the assumption that the relation between BP and CBF is linear, while physiologically CA exhibits nonlinear dynamics [9]. In this study, a new non-linear analysis method, convergent cross mapping (CCM) is applied to asses dynamic CA. Originally CCM was proposed to detect causality in complex ecosystems. According to its definition, CA can be quantified as the causal influence of BP on CBF and this causal influence can therefore be determined with CCM. Therefore the goal of this study is to explore the use of CCM in assessing dynamic CA.

METHODS

Experimental procedure

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The CCM model was validated by comparing normocapnic data with hypercapnic data. Hypercapnia causes vasodilatation of the cerebral vasculature and can therefore be used as a model for impaired CA [10]. This study included 19 healthy adults, male and female, with an age of 69 ± 4 (mean ± SD). BP was measured non-invasively in the middle finger of the right hand using photoplethysmography (FinaPres Medical Systems, Amsterdam, the Netherlands). The hand and arm were supported securely and comfortably with a sling, providing a stable position of the hand and arm at heart level. It has been shown that BP measured indirectly using the FinaPres is a reliable technique to track changes in BP that correlate well with auscultatory BP measurements in the upper arm [11]. TCD is used to measure CBF velocity (CBFV) in the middle cerebral artery (MCA) by insonating the left and right MCA using a 2 MHz TCD probe (Multi-Dop, Compumedics DWI, Germany) [12]. It is assumed that changes in CBFV represent changes in CBF, because the diameter of the vessel remains constant [10, 13]. End tidal CO_2 ($etCO_2$) was monitored with a nasal cannula using capnography (Biopac Systems, Goleta, Ca, USA). BP, CBFV and $etCO_2$ are recorded with a 200 Hz sampling frequency.

Subjects were asked to inhale a gas mixture mimicking room air, containing 0% CO_2, 21% O_2, and 79% N_2, through a tightly fitting mouthpiece until a stable plateau of CBFV had been reached. Next, the percentage of CO_2 was increased every 30 seconds, until a CO_2% of 7% was obtained. The first 90 seconds with a 0 - 2 % CO_2 concentration and the last 90 seconds with 6 - 7 % CO_2 were selected as normocapnia and hypercapnia, respectively. Beat-to-beat data of the BP and CBFV were obtained using a low pass fourth-order Butterworth filter with a cut-off frequency of 0.5 Hz. Thereafter, CBFV and BP were downsampled to a sampling frequency of 10 Hz.

Data analysis

Mathematical background of CCM

Sugihara et al. [1, 2] presented CCM as a new non-linear analysis method to determine causality between variables in a dynamical system. CCM is described in detail by Sugihara et al [1, 2]. In short, a dynamical system can be represented by a so called attractor manifold (M). Figure 1A depicts as example the manifold of the Lorentz attractor consisting of

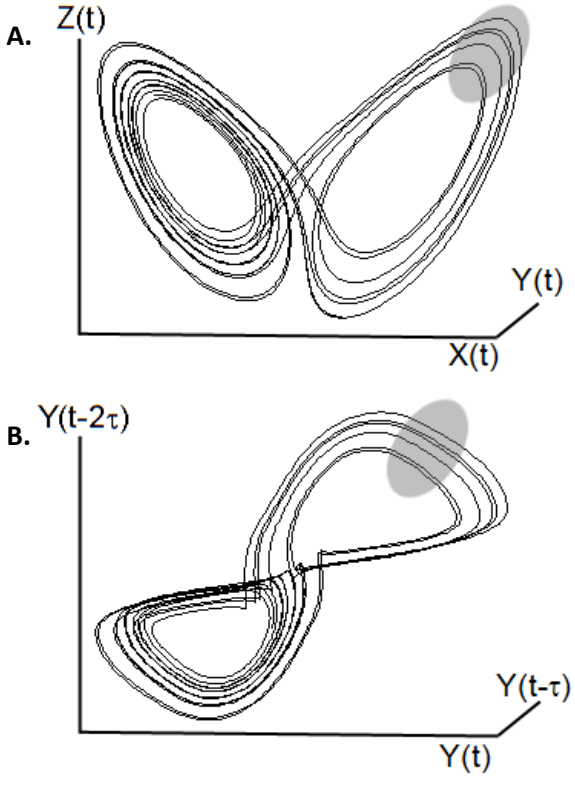

Figure 1. A) Attractor Manifold (M) of the Lorenz attractor. A point of M is defined by X(t), Y(t) and Z(t). B) Shadow manifold My with E=3 dimensions and τ = 1.4 seconds. Each point on the manifold is defined by Y(t), Y(t-τ) and Y(t-2τ). The grey area in A corresponds to the grey area in B. E = dimensions of the shadow manifold, τ = time-lag. Adapted from Sugihara et al. [2].

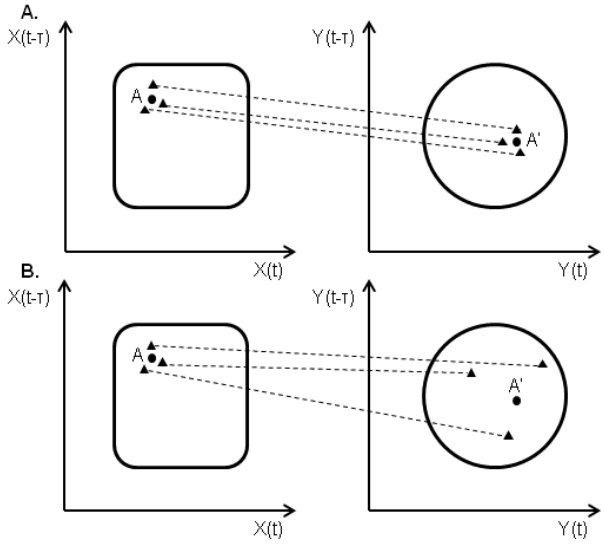

Figure 2. The nearest neighbour principle. A) Nearest neighbours (triangles) of point A (dot) on Mx are also nearest neighbours of the in time corresponding point A' on My. Therefore Mx can be used to estimate the states of Y(t), i.e. Y(t) causally influences X(t). B) Nearest neighbours (triangles) of point A (dot) on Mx are not nearest neighbours of point A' on My. Therefore Mx cannot be used to estimate the states of Y(t). Mx: shadow manifold of M with time-lagged coordinates of X(t) (E=2). My: shadow manifold of M with time-lagged coordinates of Y(t) (E = 2). E = dimension of the shadow manifold. Adapted from Sugihara et al. [1].

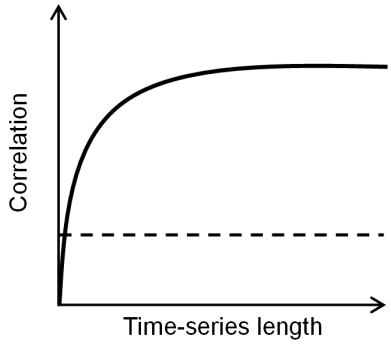

Figure 3. Principle of convergence. Solid line: Y causally influences X. Dashed line: Y does not causally influence X. The solid line shows convergence with increasing time-series length while the dashed line does not. Adapted from Sugihara et al. [1].

three variables, represented by the time-series X(t), Y(t) and Z(t). Interestingly, the dynamics of a system can also be represented using only one of the time-series, for example Y(t). Lagged coordinates of this time-series, for example Y(t-τ) and Y(t-2τ) can be used to reconstruct a shadow manifold M$_{\underline{y}}$ (Figure 1B). Tau (τ) is defined as a number of samples. M$_{\underline{y}}$ reproduces the two-lobed butterfly of M, i.e. M$_{\underline{y}}$ represents the dynamics of M. Similarly, shadow manifolds M$_{\underline{x}}$ and M$_{\underline{z}}$ can be reconstructed using X(t) and Z(t), respectively. CCM consists of two main steps that use these shadow manifolds to determine causality between variables: cross mapping and convergence.

Cross mapping

In a dynamical system, consisting of two variables (X(t) and Y(t)), cross mapping investigates if it is possible to predict a point on M$_{\underline{y}}$ from M$_{\underline{x}}$ using the nearest neighbour principle. This nearest neighbour principle is depicted in Figure 2. Point A is a random point on M$_{\underline{x}}$ and A' is the in time corresponding point of A on M$_{\underline{y}}$. The basic principle is that if nearest neighbours of A on M$_{\underline{x}}$ can accurately predict A' on M$_{\underline{y}}$, it can be stated that historical values of X(t) can be used to estimate states of Y(t). This is only possible if X(t) contains information on Y(t), in other words as Y(t) causally influences X(t). Cross mapping is applied to each point on Mx resulting in a prediction of Y(t): $Y_{Pred}(t)$. To estimate the accuracy of the $Y_{Pred}(t)$, the correlation between the $Y_{Pred}(t)$ and Y(t) is determined.

Convergence

Convergence is based on the fact that the longer the time-series length of X(t) and Y(t), the smaller the distance between the trajectories on the manifold. As a results, the estimation error decreases. Therefore, if Y(t) causally influences X(t), the correlation should increase to a plateau value with increasing time-series length, which is defined as convergence. The faster the convergence the stronger the coupling between the two variables. Figure 3 illustrates the convergence principle [1]. The cases that Y(t) does, and Y(t) does not causally influence X(t) are represented by the solid and dashed line, respectively.

Validation of CCM

CCM is applied to determine the CA quality during normocapnia (0 - 2 % CO_2) and hypercapnia (6 - 7 % CO_2). As the CA quality can be quantified as the causative effect of BP on CBFV, the shadow manifold of CBFV was used to predict BP. Generically, the shadow manifold maps 1:1 to the original manifold M. If a 1:1 mapping occurs then the shadow manifold is defined as an embedding [14]. Optimal embedding parameters, embedding dimension E and lag τ, were determined with the method of Gautama et al. [15], which is based on differential

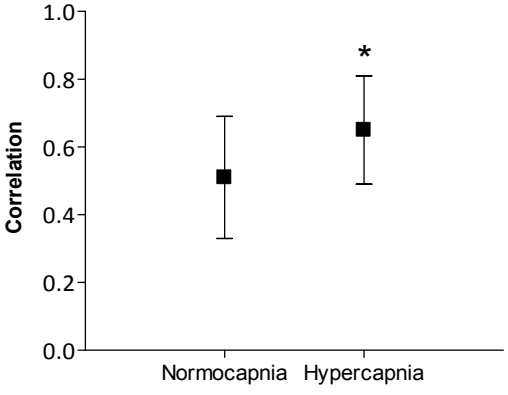

Figure 4. Correlation between real BP and predicted BP during normocapnia and hypercapnia circumstances (n=19). Correlation is significantly increased during hypercapnia (breathing 6-7% CO_2) compared to normocapnia (breathing 0-1-2% CO_2). * $p < 0.05$. BP = arterial blood pressure.

entropy. The determined optimal embedding parameters were E is 3 dimensions and τ is 1 sample. In this study, the correlation corresponding to the plateau value was used instead of the rate of convergence. A window of 890 samples was used to calculate the plateau value. Shifting the window of 890 samples through the entire dataset results in 10 correlations of which the mean is determined. The complete algorithm of CCM is described in more detail in the Supplementary materials of Sugihara et al. [1].

STATISTICS

Results are presented as means ± SD. Statistical significance was tested using a paired t-test. Significance was set at $p < 0.05$.

RESULTS

In this study, 19 healthy adults (9 males and 10 females, aged 69 ± 4 (mean ± SD)) were included. Mean BP and CBFV levels were higher during hypercapnia (BP: 114 ± 21 mmHg, CBFV: 67 ± 15 cm/s) than during normocapnia (BP: 102 ± 18 mmHg, CBFV: 44 ± 10 cm/s) ($p<<0.01$). Figure 4 depicts the correlation results for normocapnia and hypercapnia. The CCM-correlation outcome was lower during normocapnia (0.51 ± 0.18) and hypercapnia (0.65 ± 0.16) ($p = 0.01$).

DISCUSSION

Our study showed that the non-linear method CCM is able to distinguish normal dynamic CA from impaired dynamic CA. In clinical practice, the ability to measure CA may be of great importance, as impaired CA can result in hypo- or hyperperfusion of the brain. Impaired CA is also associated with increased morbidity and mortality [5]. Several methods have been developed to measure CA, however no gold standard exists. In literature, TFA is currently the most applied method to quantify CA. However, this technique assumes that CA is a linear process, while in fact CA exhibits non-linear dynamics. Zhang et al. [16] pointed out that a coherence < 0.5 in the low frequency range using TFA is an indicator of non-linear behaviour of CA. In addition, Mitsis et al. [9] showed that with the use of a non-linear model (Laguerre-Volterra network) a 20 % reduction of the normalized mean square error was seen compared to a linear model when predicting CBFV based on the input BP.

CCM is a non-linear analysis technique, which was originally proposed by Sugihara et al. [2] to detect causality in complex ecosystems. They applied CCM on a classic predator-prey dynamic system. In a classic predator-prey dynamic system, there is bidirectional causality between the predator and the prey, i.e. they both causally influence each other. The correlation converged when predicting the state of the prey using the predator data and also when predicting the state of the predator using the prey data. This indicates indeed that both factors causally influence each other. CCM was also applied on a dynamical system of sardines, anchovies and sea surface temperature. CCM showed that anchovies and sardines do not causally influence each other, but are both causally influenced by the sea surface temperature.

As CCM takes non-linear dynamics into account, this technique might also be more accurate for the quantification of CA. A well-functioning CA attenuates the effect of changes in BP on changes in CBF, i.e. BP has as only a small causal influence on CBF. During impaired CA the effect of changes in BP on changes in CBF are less attenuated, i.e. BP has a larger causal influence on CBF. Therefore the causal influence of BP on CBF is a measure of impairment of CA and CCM can be applied to assess the functioning of CA.

In this study, the ability of CCM to quantify CA was explored using a hypercapnia model. Hypercapnia is a well-known model to simulate impaired CA [17]. Hypercapnia causes vasodilatation, reducing the ability of the cerebral vessels to respond to changes in BP, leading to impaired CA. In our study a significantly higher CCM correlation value was found

during hypercapnia which indicates a less efficiently functioning CA. This underlines the potential of CCM to quantify CA.

However, still a large spread is seen in the outcome of CCM. The standard deviation was 0.2 and 0.16 for norm- and hypercapnia, respectively. Therefore, optimisation of this technique is necessary before it can be easily applied in clinical practice. There are several explanations for the large spread in CCM outcomes. First, the degree of impaired CA of each subject during hypercapnia is unknown and might differ between subjects. As a result, the spread in CCM outcome is large. However, breathing 7% CO_2 is the physiological limit. Therefore it is likely that all subjects did reach their plateau of impaired CA. Second, besides the possible difference in effect of the CO_2 on CA in subjects during hypercapnia, also the breath-to-breath $etCO_2$ fluctuations in normo- and hypercapnia circumstances between subjects might influence the correlation. Mitsis et al. [18] showed that $etCO_2$ fluctuations have a considerable effect in the lower frequencies, i.e. below 0.04 Hz. Incorporating the breath-to-breath $etCO_2$ fluctuations might therefore reduce the spread in CCM outcome.

Third, the respiratory frequency is below 0.5 Hz and because the respiratory frequency is below the cut-off frequency of 0.5 Hz, it is still present in the BP and CBFV signal. If the respiratory frequency is very constant, prediction of BP using CBFV might be easier because the fluctuations caused by respiration are then very predictable. This might result in a high CCM outcome value. On the other hand, if the respiratory frequency is less constant, prediction of BP using CBFV is harder, because the fluctuations caused by the respiration are less predictable. This results in a lower CCM outcome value. Therefore, differences in variability of the respiratory frequency between subjects might be responsible for the large spread in CCM outcome. Using a low-pass filter with a cut-off frequency 0.15 Hz might reduce the large spread in CCM outcome, because the breathing frequency is above 0.15 Hz. Because CA is most prominent in frequencies below 0.15 Hz, it can be justified to use a cut-off frequency of 0.15 Hz.

Besides the large spread in CCM outcome values, it should also be noted that in this study the plateau value was used to quantify the causal influence of BP on CBFV instead of the rate of convergence as suggested by Sugihara et al. [2]. The choice for the plateau value was based on a pilot study in which the validity of the model was investigated using the autoregulatory index of Tiecks et al. [6]. In this pilot study, the plateau value could discriminate the autoregulatory indexes. However, a situation might be possible in which the correlation does not convergence, but remains horizontal (dashed line in Figure 3). In this case, using only the

plateau value, might give inaccurate results. If this correlation is high, the plateau value falsely represents a high influence of the BP on CBFV while actually there is no influence at all. Using the rate of convergence overcomes this problem. In our study, convergence was seen in all subjects during normo- and hypercapnia. Therefore, using the plateau value was seen as a valid choice in this study. Furthermore, calculating the rate of convergence is more time-consuming than calculating the plateau value. This plateau value is therefore more promising for bedside CA monitoring. Furthermore it should be noted that the used embedding parameters were E = 3 dimensions and τ = 1 sample. These embedding parameters were determined using the differential entropy technique [15]. A τ of 1 sample is a delay of 0.1 seconds, which is within one heartbeat. It is difficult to interpret this τ physiologically, because a τ of at least one heartbeat (± 8 - 10 samples) is expected.

In summary, the ideal clinical monitoring device of CA should be non-invasive, continuous, bedside and precise. CCM is indeed a non-invasive measurement which uses spontaneous fluctuations of the ABP and CBFV to assess CA. The use of spontaneous fluctuations has the additional advantage that no interventions have to be performed, making continuously measuring CA possible. Furthermore, CCM can quantify CA using small datasets and the outcome of CCM is a single value, which is very important and practical for bedside monitoring. When the spread in CCM outcome can be reduced, perhaps with the aforementioned optimisations, CCM could be a very promising technique for future bedside monitoring of CA.

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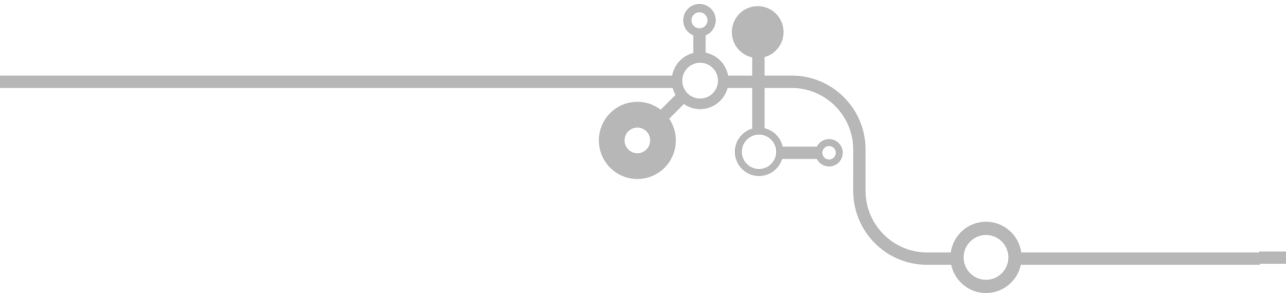

Part 2.

Clinical application of haemodynamic analysis

Chapter 6.

Impaired cerebral autoregulation and vasomotor reactivity in Alzheimer's disease.

Curr Alzheimer Res. 2014 Jan;11(1):11-7

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ABSTRACT

Background

136 Understanding the relationship between vascular disease and Alzheimer's disease (AD) will enhance our insight into this disease and pave the way for novel therapeutic research. Cerebrovascular dysfunction, expressed as impaired cerebral autoregulation and cerebral vasomotor reactivity, has been observed in transgenic mouse models for AD. Translation to human AD is limited and conflicting however.

Objective

To investigate if impaired cerebral autoregulation and cerebral vasomotor reactivity, found in animal models for AD, are present in human sporadic AD.

Methods

In 12 patients with mild to moderate AD (age 75 ± 4 yr) and 24 controls matched for age and history of hypertension, all without diabetes, we measured blood pressure (FinaPres) and cerebral blood flow-velocity (transcranial Doppler). Cerebral autoregulation was assessed during changes in blood pressure induced by single and repeated sit-stand manoeuvres. Cerebral vasomotor reactivity was assessed during hyperventilation and inhalation of 5 % carbon dioxide.

Results

During single sit-stands, controls had a 4 % (SD 8) decrease in cerebrovascular resistance during a reduction in blood pressure and an 8 % (SD 11) increase during a rise in blood pressure, indicating normal cerebral autoregulation. These changes were not seen in AD ($p=0.04$). During repeated sit-stands, blood pressure fluctuated by 20 % of baseline. This led to larger fluctuations in cerebral blood flow in AD (27 (6) %) than in controls (22 (6) %, $p < 0.05$). Cerebral vasomotor reactivity to hypercapnia was reduced in AD (42.7 % increase in CBFV, versus 79.5 % in controls, $p = 0.03$).

Conclusion

Observations of impaired cerebrovascular function (impaired autoregulation and vasoreactivity) in transgenic mouse models for AD were confirmed in patients with sporadic AD.

INTRODUCTION

Alzheimer's disease (AD), the leading cause of dementia, is a progressive neurodegenerative disorder. Worldwide, AD is highly prevalent and places a huge burden on patients and caregivers [1, 2]. While the number of AD patients grows, there remains limited understanding of this disease and its underlying causes. This is reflected in the lack of an effective curative treatment for AD.

A large body of recent evidence suggests a strong link between AD and vascular disorders and vascular risk factors [3-6]. The exact relationship however between these factors and AD remains poorly understood. Better understanding of how vascular factors relate to AD may therefore enhance our insight into this disease and pave the way for novel therapeutic research.

Research into the vascular pathophysiology of AD can be categorized in two hypothesized 'causal directions', one direction wherein vascular disease leads to (or promotes) Alzheimer pathology, and another, opposite, direction wherein Alzheimer pathology causes vascular disease. These two 'directions' are likely to interact, as is exemplified by cerebral autoregulation.

Cerebral autoregulation (CA) is the mechanism that aims to maintain a stable cerebral blood flow in the event of a change in blood pressure (BP) [7, 8]. Evidence that AD is associated with severe impairment in CA was first observed by Niwa et al. (2002) [9]. In this study, mice carrying a human AD mutation were unable to preserve cerebral blood flow when their BP was lowered. This evidence was corroborated by studies showing *in vitro* impairment in cerebrovascular function and structure in models for AD [9, 10]. Although this is an example of the vascular hypothesis direction wherein AD leads to vascular disease, the possible consequences of this impairment in CA serve to illustrate the complex interaction between the two directions: the impairment in CA (due to vascular changes brought about by AD) increases the risk of cerebrovascular insufficiency (e.g. ischemia, hypoperfusion) which in turn may contribute to the progression of AD [11].

In addition to an impairment in CA, evidence also suggests that cerebral vasomotor reactivity (CVMR) is affected in AD. CVMR is a mechanism that reflects the uniquely strong response of cerebral blood vessels to changes in arterial carbon dioxide concentration [12]. Two different mouse models for AD demonstrated cerebral microvascular impairment in

vasomotor reactivity to hypercapnia [10, 13].

Whether these animal model findings of impaired CA and CVMR can be translated to human AD remains uncertain [9]. The aim of this study was to assess CA and CVMR in patients with AD and age-matched controls, with the hypothesis that CA and CVMR both show impaired vasodilatatory responses in human AD, comparable to observations in AD animal models.

MATERIALS AND METHODS

Study Population

We studied 12 patients with mild to moderate AD (age 75 ± 4 yr) and 24 controls matched for age and – because hypertension is a common comorbidity in AD – also for history of hypertension. None of the AD patients or controls had diabetes, however. All subjects were carefully screened by a geriatrician including a medical history, physical examination, and electrocardiogram to exclude acute medical conditions or cardiovascular diseases other than hypertension. Patients with AD were diagnosed as probable AD by a multidisciplinary memory clinic team using the NINCDS-ADRDA criteria [14, 15] based on clinical evaluation and additional diagnostic tests, such as MRI-scan of the brain (which was consistent with mild global atrophy combined with hippocampal atrophy and no or minimal cerebrovascular disease), neuropsychological testing (which showed a predominant disorder in episodic memory) , and cerebrospinal fluid analysis (available in n = 4, showing a biomarker profile consistent with AD). Informed consent was obtained from patients and their proxy [16], and from controls, before they entered the study. The study was approved by the medical ethical committee.

Data Acquisition

BP was measured continuously and noninvasively in the middle finger of the right hand using FinaPres (FinaPres Medical Systems, the Netherlands). The hand and arm were supported securely and comfortably with a sling, providing a stable position of the hand and arm at heart level throughout the whole measurement. It has been shown that BP measured indirectly in a finger by photoplethysmography is reliable in assessing changes in BP that correlate well with auscultatory BP measurements in the upper arm [17].

Cerebral blood flow velocity (CBFV) was obtained in the middle cerebral arteries (MCA) by

transcranial Doppler ultrasonography. The left and right MCA were insonated by placing a 2-MHz Doppler probe (Multi-Dop, Compumedics DWL, Germany) over the temporal window. The MCA were identified according to their signal depth, velocity and wave characteristics [18]. If only one signal was available due to one-sided temporal window failure, we included this available signal for analysis. The probes were locked at a constant angle and position during data collection with a customized headband (Spencer technologies, Seattle, Wa.).

End-tidal CO_2 ($etCO_2$) was monitored with a nasal cannula using capnography (BIOPAC Systems, Goleta, Ca.).

Experimental Procedure

All experiments were performed in the morning, at least 2 h after a light breakfast and 12 h after the last caffeinated beverage or alcohol, in a quiet, environmentally controlled laboratory with an ambient temperature of 22°C. After at least 10 min of rest in sitting position, a baseline measurement of 5 min was recorded during spontaneous respiration.

To assess CA, subjects were asked to perform a single sit-to-stand protocol. Subjects sat in a straight-backed chair and were asked to stand up. The protocol consisted of three trials of 2-min sitting followed by standing for 1 min. After this, repeated sit-stand manoeuvres were performed [19]. Patients were coached into performing these manoeuvres at a frequency of 0.05 Hz (10 s sitting, 10 s standing) for 5 min. During all manoeuvres, subjects were instructed to keep normal breathing and to avoid performing a Valsalva manoeuvre. Adherence to this was confirmed by inspecting the CO_2 waveforms and $etCO_2$ registrations.

To estimate CVMR a previously described protocol was used [20], consisting of a 30 s period of coached hyperventilation, followed by 2 minutes of spontaneous breathing. Next, subjects were asked to inhale a gas mixture containing 7 % CO_2, 21 % O_2, and 72 % N_2 through a tightly fitting mouthpiece until a stable plateau of CBFV had been reached. With this protocol a wide range of changes in etCO2 can be obtained.

Data Processing

All data were simultaneously recorded at 200 Hz. Post processing was performed using custom-written MATLAB scripts. Real time beat-to-beat mean values of BP and CBFV were

calculated as waveform integration of the BP and CBFV signal within each cardiac cycle.

The CVMR protocol evaluated changes in CBFV, BP, and calculated cerebrovascular
140 conductance index (CVCI), during the transitions from hypocapnia (induced by hyperventilation) to normocapnia and from normocapnia to hypercapnia (induced by 7 % CO_2 inhalation).

Because changes in CO2 cause changes in BP, which in turn may directly affect CBFV, CVCI, expressed as CBFV changes divided by BP changes, was used to minimize the confounding effects of differences in BP response during CVMR testing between subjects on CVMR estimation [21].

For CA, to evaluate the beat-to-beat dynamics of BP and CBFV responses to acute posture changes in the single sit-stand protocol, we calculated the differences between the sitting value (averaged over a period of 20 s) and the value at the nadir of BP (average of 5 values surrounding the nadir) for BP and CBFV.

The repeated sit-stand manoeuvres were evaluated by calculating the differences between the maximal sitting value (average of 5 values surrounding the top) and the value at the nadir of BP after standing (average of 5 values surrounding the nadir) for BP and CBFV for each sit-to-stand manoeuvre.

This way only the amplitude relationship between changes in BP and CBFV was taken into account, the phase shift between BP and CBFV was disregarded. In addition to these absolute values, we also expressed these changes as percentage of baseline. The average of the trials for a group was then computed.

Finally, we determined the transfer function analysis (TFA) from the spontaneous oscillations in the baseline measurement as a measure for CA using the method described by Zhang et al. (1998) and reviewed in [8]. The time series of mean BP and CBFV were first subdivided into 950-point segments with 50% overlap for spectral estimation. This process resulted in five segments of data for the segment periodogram average. Fast Fourier transforms were implemented with each Hanning-windowed segment and averaged to quantify the transfer function. For quantification of CA the positive phase shift and the gain between BP and CBFV were quantified as means of the following frequency bands: very low frequency (VLF): 0.02 - 0.07 Hz; low frequency (LF): 0.07 - 0.15 Hz.

The TFA is based on the high-pass filter model of cerebral autoregulation, wherein BP oscillations at lower frequencies are buffered better than higher frequencies, leading to lower gain (better damping) in these lower frequencies. Also, a characteristic of this model is that the counteractive actions of autoregulation lead to a phase shift between CBF and BP in these lower frequencies [8].

STATISTICAL ANALYSIS

Results are presented as the means ± standard deviations (SD) or percentages unless otherwise stated. Differences in variables between patients and controls were evaluated with the independent student t-test for continuous variables and the chi-squared (χ^2) statistic for proportions. The results were considered to be significant for p-values < 0.05.

RESULTS

Subject Characteristics

Demographic and baseline data for the 12 AD patients and the 24 controls are summarized in Table 1. AD and controls were well matched for age and history of hypertension, however there was more use of anti-hypertensive medication in AD (p = 0.01), and BP was somewhat higher at baseline (although only significant for diastolic BP, p = 0.03). Previous studies [22-24] have shown higher cerebrovascular resistance in AD. This trend was observed here but did not reach significance likely due to the small sample and high intra-individual variability.

Cerebral Vasomotor Reactivity

One healthy control could not perform the CVMR procedure adequately and was excluded from this analysis. Table 2 summarizes the haemodynamic results for CVMR.

AD patients and controls had similar $etCO_2$ values for baseline and for the minimum and maximum $etCO_2$ levels reached during hyperventilation and CO_2 breathing (p > 0.05, Table 2). Changes in BP were also similar in both groups during normocapnia, hyperventilation and CO_2 breathing. Changes in CBFV did not differ during baseline or hyperventilation, however during 7 % CO_2 breathing CBFV increased less in AD (p < 0.01). This difference in the CBFV response over the full range from hypocapnia to hypercapnia between AD and controls is also

142 **Table 1.** Baseline characteristics.

All values are mean (SD). AD= Alzheimer's disease patients. MMSE= Mini Mental State Examination. CAMCOG = Cambridge cognitive examination (higher scores are better, cut-off scores are age and education-dependent but usually lie between 77-84). CDR: clinical dementia rating scale, 0 = normal, 0.5 = mild cognitive impairment, 1 = mild dementia, 2 = moderate and 3 = severe dementia. BP = mean arterial blood pressure, SBP= systolic and DBP = diastolic blood pressure. CBFV= mean cerebral blood flow velocity, CV resistance = cerebrovascular resistance (CBFV/BP). †significantly different ($p<0.05$) from control.

	AD patients (n = 12)	**Controls (n = 24)**
Age (years)	74 (4)	76 (4)
Sex (male/female)	9-3	18/6
MMSE (0-30)	22 (5) †	29 (1)
CAMCOG (0-104)	69 (13)	NA
CDR (0-3)	0.9 (0.2)	0
Hypertension (n(%))	6 (50)	9 (38)
SBP (mmHg)	140 (33)	127 (19)
DBP (mmHg)	69 (13) †	58 (9)
BP (mmHg)	105 (24)	100 (11)
CBFV (cm/s)	45 (15)	50 (11)
CV resistance (mmHg*s/cm)	2.6 (1)	2.1 (0.6)
Medication use		
Beta blocker	4/12	3/24
ACE inhibitor	2/12	0/24
ARB	0/12	0/24
Calcium channel blocker	1/12	0/24
Thiazide diuretics	3/12 †	0/24

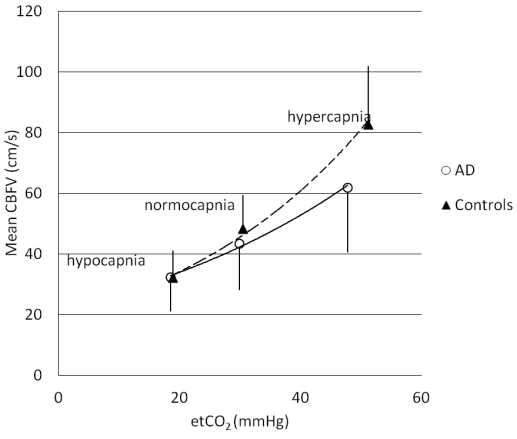

Figure 1. Cerebral vasomotor reactivity.

Mean cerebral blood flow-velocity (CBFV) changes induced by hyperventilation (hypocapnia) and CO_2 breathing (hypercapnia) in AD patients and healthy controls. Solid line: controls. Dashed line: AD.

Table 2 .Cerebral vasomotor reactivity results.

All values are mean (SD). See legend table **1**. CVCI= cardiovascular conductance index (BP/CBFV), $etCO_2$ =end-tidal CO2. † significantly different ($p<0.05$) from control.

Hypocapnia (Hyperventilation)	**AD patients (n = 12)**	**Controls (n = 23)**
BP (mmHg)	94 (33)	93 (15)
CBFV (cm/s)	32(12)	32 (9)
CVCI (cm/s/mmHg)	0.32 (0.1)	0.35 (0.1)
$etCO_2$ (mmHg)	19 (6)	19 (6)
Normocapnia	**AD patients (n = 12)**	**Controls (n = 23)**
BP (mmHg)	118 (25)	111 (15)
CBFV (cm/s)	44 (16)	48 (11)
CVCI (cm/s/mmHg)	0.38 (0.1)	0.44 (0.1)
$etCO_2$ (mmHg)	30 (5)	31 (5)
Hypercapnia (CO_2 breathing)	**AD patients (n = 12)**	**Controls (n = 23)**
BP (mmHg)	139 (33)	138 (21)
CBFV (cm/s)	62 (22) †	83 (19)
CVCI (cm/s/mmHg)	0.46 (0.2) †	0.60 (0.1)
$etCO_2$ (mmHg)	48 (9)	51 (6)

shown in Figure 1. CVMR was decreased in AD for hypercapnia (42.7 % increase in CBFV, versus 79.5 % in controls, p = 0.03) but not for hypocapnia (30.0 % decrease in CBFV, versus 32.4 % in controls, p = 0.2). Over the full range of etCO2, the CVMR was also clearly reduced in AD (controls 111.6 %, AD 73.2 %, p < 0.01). Expressed as a ratio of CBFV changes over changes in $etCO_2$, CVMR was also lower in AD compared to controls (1.09 ± 0.39 vs. 1.44 ± 0.43 $cm.s^{-1}.mmHg^{-1}$, p = 0.02).

Cerebral Autoregulation: Cerebral Blood Flow Responses to Single Step Changes in Blood Pressure

The haemodynamic changes for the single sit-stand manoeuvres are summarized in Table 3. The posture change from sit to stand evoked transient reductions in BP and CBFV, but did not result in a difference between AD and control in either the maximum reduction in BP, the maximum reduction in CBFV, or the time to nadir.

Table 3. Sit-stand manoeuvre.

All values are mean (SD). See legend table 1. tBP nadir = time from standing up to the nadir of blood pressure. For stand-to-sit, this was the time from sitting down to the maximum increase in blood pressure. † = significantly different (p<0.05) from control.

	Single Sit-to-Stand		Single Stand-to-Sit	
	AD patients (n = 12)	Controls (n = 24)	AD patients (n = 12)	Controls (n = 24)
Absolute change				
BP (mmHg)	-18 (5)	-20 (6)	15 (5)	13 (6)
CBFV (cm/s)	-9 (6)	-8 (2)	7 (4) †	4 (3)
CV-Resistance (mmHg*s/cm)	0.06 (0.3) †	-0.1 (0.1)	-0.02 (0.2)	0.10 (0.2)
Percentage change				
BP (%)	-18 (7)	-21 (7)	21 (11)	21 (10)
CBFV (%)	-18(8)	-17 (5)	24 (15) †	13 (9)
CV-Resistance (%)	3 (7)	-4 (8)	-0.8 (9)†	8 (11)
tBP nadir (s)	11 (6)	11 (3)	5 (0.8)	6 (2)

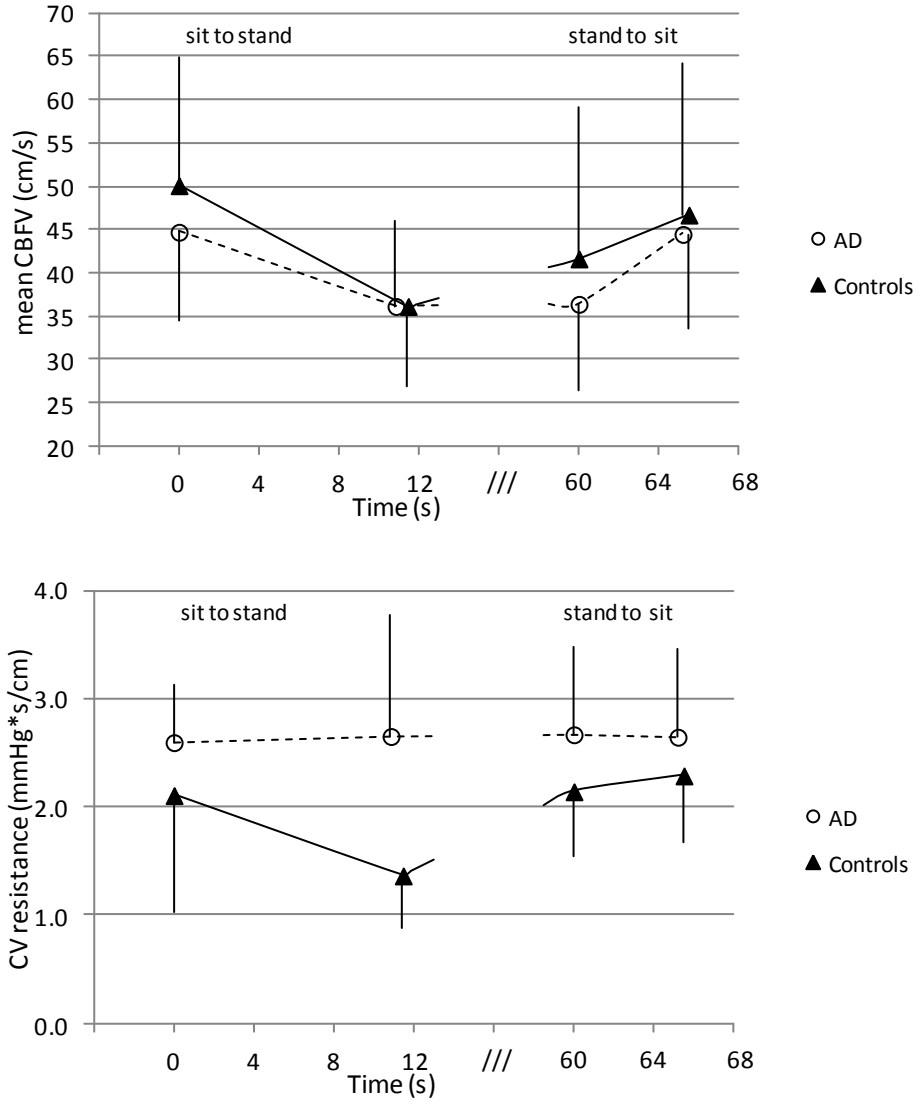

Figure 2. Sit-stand manoeuvre.

Mean cerebral blood flow-velocity (CBFV) changes (top) and cerebrovascular resistance index (bottom) induced by single sit-stand and stand-sit manoeuvre in AD patients and healthy controls. Sit-stand: standing up after 2 min sitting. Stand-sit: return to sitting after 1 min standing. Solid line: controls. Dashed line: AD.

Table 4. Maximal changes caused by repeated sit-stand procedure.

All values are mean (SD). See legend table 1. [†]significantly different ($p<0.05$) from the control group.

	AD patients (n = 9)	Controls (n = 24)
Absolute change		
BP (mmHg)	20 (4)	21 (6)
CBFV (cm/s)	11 (4)	11 (5)
Percentage change		
BP (%)	20 (4)	21 (4)
CBFV (%)	27 (6) [†]	22 (6)

However, normal CA, in an attempt to restore CBF to baseline following a reduction in BP, will cause a reduction in cerebrovascular resistance. Despite similar proportions of changes in BP and CBFV, this expected normal reduction in cerebrovascular resistance was observed in controls but not in AD (difference: -0.06 mmHg*s/cm, $p=0.04$, see Table 3). The posture change from standing back to sitting led to similar increases in BP. Also here, the expected increase in CVR when CA attempts to buffer this increase in BP was not observed in AD. As a result, the proportional increase in CBFV for a similar increase in BP was larger in AD ($p = 0.02$; Table 3, Fig. 2). Together, these results show impaired responses in cerebrovascular resistance in AD, indicating diminished CA.

Cerebral Autoregulation: Cerebral Blood Flow Responses to Repeated Changes in Blood Pressure

Average BP and CBFV over time during the repeated sit-stand procedure are shown in Figure 3. Three AD patients were not able to perform the repeated sit-stand procedure, due to reduced motion and balance control, and impaired physical fitness, and were excluded from this analysis. The maximum haemodynamic changes caused by the repeated sit-stands are reported in Table 4. The repeated sit-stand manoeuvres caused similarly large perturbations in absolute and relative (percentage) changes in BP ($p > 0.3$) in AD and controls. However, the relative changes in CBFV were higher in AD (27 ± 6 %) than in controls (22 ± 6 %) ($p = 0.03$), suggesting less effective damping by cerebral autoregulation.

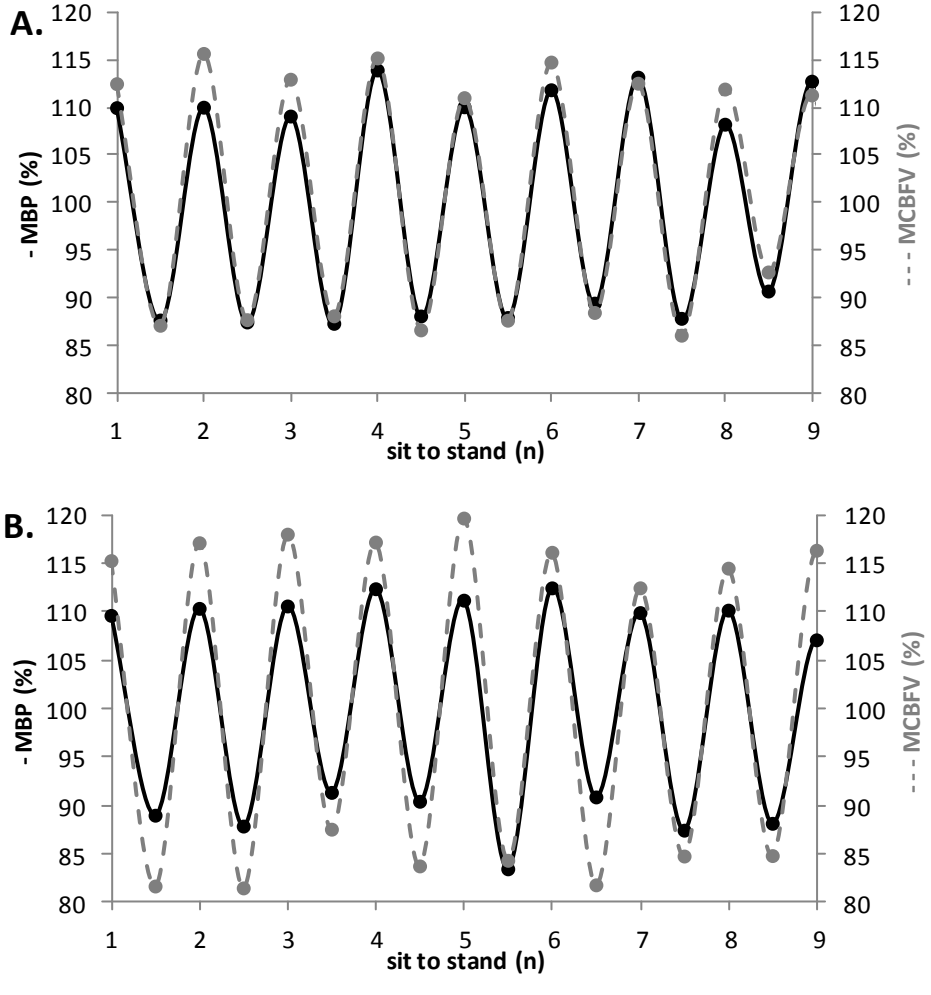

Figure 3. Repeated sit-stand manoeuvre.

Average percent changes in mean arterial blood pressure (BP, black lines) and cerebral blood flow-velocity (CBFV, grey dashed lines) for the repeated sit- stand procedure for controls (A) and AD (B). 8 consecutive repetitions of 10 s standing followed by 10 s sitting are shown. X-axis represents the number of sit-stands. Note that this graphical representation only shows differences in amplitude, the temporal relationship between BP and CBFV, including any phase shift cannot be determined in this way.

Cerebral Autoregulation: Transfer Function Analysis

A comparison between AD and controls based on the parameters derived from TFA (phase and gain in the very low, and low frequency ranges of spontaneous oscillations in BP and CBFV) revealed no differences. VLF: phase AD 0.88 ± 0.3 rad, phase controls 0.76 ± 0.3 rad, $p > 0.05$; gain AD 0.39 ± 0.2 cm/s/mmHg, gain controls 0.47 ± 0.1 cm/s/mmHg $p > 0.05$. LF: phase AD 0.76 ± 0.3 rad, controls 0.73 ± 0.3 rad, $p > 0.05$; gain AD 0.64 ± 0.4 cm/s/mmHg, controls 0.63 ± 0.2 cm/s/mmHg, $p > 0.05$.

DISCUSSION

The main finding of this study is that patients with AD demonstrated impairments in CA and CVMR that were similar to observations of cerebrovascular dysfunction in transgenic mouse models for AD. AD patients had a blunted vasodilatatory response to hypercapnia (impaired CVMR) as well as insufficient vasodilatatory and vasoconstrictor responses to decreases and increases in BP (impaired CA).

Preclinical research suggests that Alzheimer pathology affects vascular function [9-11, 13, 25]. This research involved mouse models for AD and *in vitro* observation of animal and human vascular cells, such as endothelial cells. Post-mortem studies confirm that there are cerebrovascular changes, related to amyloid pathology, in human AD, including degenerated string capillaries and reduced markers linked to vasoconstriction and vasodilatation [26]. Together, this work implies that adaptive cerebrovascular responses may be impaired in AD, such as the cerebral vasodilatatory response to hypotension (cerebral autoregulation), or to increased neuronal demand for oxygen (neurovascular coupling), see [11, 25]for review. Such impaired responses would represent an increased risk of insufficient blood supply to the brain in a patient with AD and could accelerate cognitive decline. It is therefore essential to understand if these impaired cerebrovascular responses are observed not only in animal or *in vitro* human models, but also in humans known to have AD.

Such translational evidence was limited thus far, however. To date, five studies had investigated CA in human AD [23, 24, 27-29], for review see [11], but found no evidence for impairment of CA. Four of these studies investigated dynamic CA (the CBF response to fluctuating changes in BP), using transcranial Doppler to measure CBF [23, 24, 27, 28]. All three used transfer function analysis (TFA) to assess CA [7, 8]. This method may be less sensitive to detect more subtle impairment in CA in small samples. This is supported by the

fact that also in the present study TFA did not reveal a difference in autoregulation between AD patients and healthy controls. TFA combines responses to BP increases and decreases, and does not consider each direction separately. The suggestion that TFA may be less sensitive is further supported by the fact that two of the studies that used TFA also investigated changes in cortical oxygenation and found that frontal cortical oxygenation changes in response to BP changes were stronger in AD, suggesting microvascular impairment, despite normal findings on TFA [27, 28].

The fifth study investigated static CA (the CBF response to a steady-state reduction in BP, comparable to the experiment Niwa et al. performed in mice) using PET to measure CBF [29]. No differences were observed between AD and controls, however, the response to increases in BP was not tested and a calcium-channel blocker was used to lower BP, and this may have supported CA by promoting cerebral vasodilatation [9].

Regarding CVMR, two previous studies have investigated CVMR in AD, and confirmed our observation of impaired CVMR [22, 30].

Our study adds to these seven earlier studies by combining CA and CVMR assessment in the same patients. In addition, the method we used to measure CA was more straightforward and therefore perhaps more sensitive to changes than transfer function analysis. CA operates by adapting cerebrovascular resistance to changes in BP [8]. We investigated these changes in cerebrovascular resistance during reductions and increases in BP induced by changes in posture, thus avoiding pharmacological interventions to change BP. We show that these changes in cerebrovascular resistance were reduced in AD, suggesting impaired ability to dilate and constrict. In addition, we challenged CA by inducing repeated increases and decreases in BP, and this led to larger fluctuations in CBF in AD. Again, this suggests that CA is less able to dampen these changes because vasodilatation and constriction are impaired.

While CA assesses vasodilatation and constriction in response to changes in BP, CVMR assesses these responses to changes in CO_2. AD patients had reduced vasodilatation to hypercapnia, but vasoconstriction to hypocapnia was not affected. In summary, AD patients had impaired vasodilatation and constriction responses to changes in BP, and impaired vasodilatation responses to hypercapnia.

This is an important translational finding [9, 10, 13]. Criticism of transgenic animal models for AD is that they are not representative for the majority of AD patients who have sporadic AD

(i.e. who do not have genetic mutations leading to AD). Our observation of similar cerebrovascular dysfunction in human sporadic AD and transgenic mouse models leads to the following considerations. First, it can be argued that the observations of impaired CA and
150 CVMR in our patients are caused not by AD but by comorbid vascular disorders that may be more prevalent in AD. Indeed, even though we tried to match for hypertension, as in many other studies AD patients had higher BP. However, the animal models find similar cerebrovascular dysfunction, in the absence of confounding human vascular comorbidity. This comparison supports the interpretation that the observed changes in CA and CVMR are related to AD. Second, the animal models link impaired CA and CVMR to cerebral amyloid angiopathy, reflecting vascular deposition of amyloid-beta [9, 13]. The impairment in CVMR in AD was similar to that observed in patients with cerebral amyloid angiopathy [30] and compares to the impairment of CVMR in the transgenic models. Third, the impairments in CA and CVMR are in line with post-mortem observations in AD that found cerebrovascular changes that were suggested to cause impairments in dilatation and constriction [26]. Taken together, this evidence suggests that the cerebrovascular impairments observed in animal models for AD are also observed in sporadic AD, and may both be explained by vascular effects of amyloid-beta.

Limitations of this study are the small sample size. However, despite this small sample, the differences in CA and CVMR were already obvious. Second, the diagnosis of AD remains a clinical one. No pathological confirmation of the underlying disease was available. Still, previous studies have shown that a clinical diagnosis is only inaccurate in about 11 % of mild cases when compared to pathological diagnosis [31]. AD was diagnosed by a multidisciplinary memory clinic team consisting of several geriatricians, neuropsychologists, occupational therapists and speech therapists. Lewy body dementia and vascular dementia were excluded based on diagnostic criteria for these conditions, using information obtained by means of history, clinical examination, laboratory tests and MRI.

Finally, the clinical implications of the observed impairments in CA and CVMR are unclear. The impairment in CA is clear but subtle, and did not lead to severe hypoperfusion, and may not lead to cerebral ischemia. Indeed, we recently found no increased susceptibility to white matter lesions in AD [32]. It is not unthinkable however that chronic mild hypoperfusion contributes to brain atrophy in AD. Impaired CVMR may affect neurovascular coupling, which may contribute to cognitive dysfunction [25].

CONCLUSION

AD patients have impaired cerebral autoregulation, leading to reduced ability to stabilize CBF during changes in BP, as well as impaired CVMR, which leads to much smaller increases in CBF with hypercapnia. These findings are similar to observations in transgenic animal models for AD, where impaired CA and CVMR are thought to be induced by amyloid pathology, especially perivascular amyloid depositions. These translational findings support the vascular hypothesis for AD, specifically the direction where AD causes vascular dysfunction.

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Chapter 7.

Baroreflex function is reduced in Alzheimer's disease: a candidate biomarker?

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ABSTRACT

158 The baroreflex (BR) reflects autonomic blood pressure control. Alzheimer's disease (AD) affects the autonomic system. Detailed properties of BR in AD are unknown. We hypothesized that BR is reduced in AD, and is influenced by autonomic effects of cholinesterase inhibitors (ChEI). BR was determined in 18 AD patients, 11 patients with mild cognitive impairment (MCI) and 19 healthy control subjects. In AD, BR was measured again after ChEI treatment. Receiver operating characteristic analysis was used to define a BR cut-off value, which was then tested in an independent validation sample of 16 AD, 18 MCI, and 18 control subjects. BR was lower in AD compared with MCI ($p < 0.05$) and in MCI compared with healthy control subjects ($p < 0.01$). Receiver operating characteristic analysis between AD and healthy control subjects yielded a sensitivity of 89 % and a specificity of 94 %. ChEI treatment increased BR with 66 % ($p < 0.01$). BR was reduced in AD and increased after treatment with ChEI. BR might be a good biomarker to further explore the link between cardiovascular disease and AD.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is the leading cause of dementia. Worldwide, AD is among the top 5 of most costly diseases and places a huge burden on individual caregivers [2]. There is still a limited understanding of this disease and its underlying cause, which is reflected in the lack of an effective curative treatment for AD.

Many of the currently developed and tested therapies are based on the amyloid cascade hypothesis. This hypothesis, supported by convincing genetic data, proposes that the extensive neuronal damage in AD is caused by increased concentration and aggregation of β-amyloid [3]. However, this theory does not offer an explanation for how this process is initiated in sporadic forms of AD, nor for the observation that the earliest changes in AD do not include amyloid deposition [4]. Criticism regarding the amyloid hypothesis has fuelled the popularity of the vascular hypothesis for AD, which states that cardiovascular disease is an important causal or contributing factor in sporadic AD [5-8].

Although cardiovascular factors are now commonly accepted as risk factors for AD, the exact relationship between these factors and AD remains poorly understood. For example, midlife hypertension increases AD risk and antihypertensive treatment potentially reduces this risk [9, 10], suggesting an unidirectional, possibly causal relationship between hypertension and AD. However, subjects with a parental history of sporadic AD are more likely to have hypertension than control subjects [11], suggesting either a causal relationship between AD and hypertension or a shared causative factor. Moreover, blood pressure (BP) levels decline from hypertension to normotension or even to hypotension during the course of AD [12]. These data imply a relationship wherein AD might influence BP or BP might influence AD. Better understanding of the relationship between BP control and AD might therefore provide important new clues toward our understanding, and ultimately, treatment, of AD.

A possible link between AD and BP is the baroreflex (BR). The BR is a reflex loop with cardiac, vascular, and cerebral components involved in short-term BP regulation [13]. The BR operates via the autonomic nervous system to restore sudden changes in BP by changing heart rate (HR) or vascular tone. A clinical example is the drop in BP on standing, which the BR corrects by a rapid increase in HR (parasympathetic inhibition) followed by peripheral arterial vasoconstriction (sympathetic activation). The cholinergic system is an important component of cardiovascular and autonomic control, including the BR. This cholinergic system is prominently affected early in AD [14, 15]. Therefore, we hypothesized that BR function is

reduced early in patients with AD [16]. In this study, we further explored this hypothesis in 3 steps. First, we compared BR function between patients with AD, patients with mild cognitive impairment (MCI), and healthy elderly subjects. Patients with MCI are thought to represent patients with very early-stage AD. The second step was to validate our findings in an independent sample of AD patients, MCI patients, and elderly control subjects. The third and final step was to explore the role of the cholinergic deficit on BR function in AD, by testing the influence of cholinesterase inhibitors on BR function in a subgroup of patients with AD.

METHODS

Study population

Overall, this study included 34 patients with mild to moderate AD, 29 patients with MCI and 37 healthy control subjects in 2 distinct samples: (1) a derivation sample: 18 patients with AD (72 ± 6 years), 11 patients with MCI (71 ± 9 years), and 19 healthy control subjects (75 ± 3 years), recruited at Radboud University Nijmegen Medical Centre (RUNMC), and (2) a validation sample: 16 AD patients (71 ± 8 years), 18 patients with MCI (70 ± 7 years), and 18 healthy control subjects (70 ± 6 years) recruited at Maastricht University Medical Center (MUMC). Data on cerebral hemodynamics from the RUNMC AD patients and control subjects and from the MUMC AD and MCI patients and control subjects have been published recently [17, 18].

In both samples, recordings of electrocardiograms (ECG) and beat-to-beat photo-plethysmographic BP were obtained. There was a difference in body position during measurements between the 2 samples. Subjects at RUNMC were seated while ECG and BP were recorded, whereas the subjects at MUMC were supine.

All subjects from both centres were examined by a geriatrician, and carefully screened to exclude acute medical conditions. A subset of subjects (15 control subjects; 15 MCI patients; 29 AD patients) had a magnetic resonance imaging scan of the brain to investigate medial temporal lobe atrophy. Informed consent was obtained from all patients and healthy control subjects before they entered the study. Both studies were approved by the medical ethical committees of the corresponding centres.

Patients with MCI and AD were diagnosed by a multidisciplinary memory clinic team using the

National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria [19, 20] and MCI consensus criteria [21] based on clinical evaluation and additional diagnostic tests if indicated, such as magnetic resonance imaging scan of the brain, neuropsychological testing, and cerebrospinal fluid analysis. In the derivation sample from RUNMC, BR in AD patients was measured before and after 8 weeks of treatment with a cholinesterase inhibitor (galantamine, 4 weeks of 8 mg followed by 4 weeks of 16 mg). In the validation sample from MUMC, 10 of the 16 AD patients were already treated with cholinesterase inhibitors when BR was measured.

Data acquisition and pre-processing

The ECG was recorded using a 3-lead system and beat-to-beat HR was obtained from each R-R interval identified from the QRS complexes. Arterial BP was measured noninvasively at the middle finger of the right hand using FinaPres (FinaPres Medical Systems) at RUNMC and the Task Force Monitor (CN Systems) at MUMC. It has been shown that BP measured on the finger by photoplethysmography is similar to conventional auscultatory measurements on the upper arm [22]. For both systems, the finger pressure cuff was positioned carefully at heart level with the hand being held in the left midaxillary line. The hand and arm were supported securely and comfortably with a sling. The subjects underwent several minutes of customization and the servo-adjust mechanism was turned off before recording. Periods of 100 seconds of artefact- and calibration-free data were selected by visual inspection of the time series of the tachogram and systogram and used for subsequent analysis. The time series were linearly interpolated at 1 Hz to obtain equidistant time intervals. The time series were detrended and filtered with an eighth-order high-pass Butterworth filter (0.02 Hz), to ascertain signal stationarity.

Causal model BR estimation

BR sensitivity is often evaluated noninvasively by assessing heart rate variability (HRV) and systolic blood pressure (SBP) variability. However, studies using HRV and SBP variability often report contradictory results [23-25]. A possible explanation for this inconsistency is that the estimation of the BR by means of HRV assumes but does not test that the whole R-R interval (RRi) variability is generated by SBP changes. Therefore the causal dependencies are not taken into account. In this study, a bivariate causal model (ARXAR model), which was first introduced by Nollo et al. [1], was used to quantify the BR. With this model it is possible to describe the causal relationship from SBP to R-R interval (the BR pathway) and to separate it

from the mechanical pathway (from RRi to SBP). Details of this method are described elsewhere [1].

162 In short, the interactions between RRi and systolic blood pressure (SBP) are modelled as follows:

$$RRi(n) = -\sum_{k=1}^{p} a_k RRi(n-k) + \sum_{k=1}^{p} b_k SBP(n-k) + u(n)$$

According to the ARXAR model, the RRi is affected by p samples of its own past (by ak coefficients) and by p samples of the SBP sequence (by bk coefficients). The effects of other sources independent from SBP on RRi variability, considered as noise in this context, are accounted for in the model by means of the u(n). As outlined in Figure 1, the SBP and u signals are described as autoregressive processes with w_{sbp} and w_{rri} zero-mean input white noises.

The blocks C and D are formed by the autoregressive parameters of SBP and u, respectively. In the open loop ARXAR model the variability of SBP around its mean value is considered as an exogenous input, i.e. it may affect the RRi variability without itself being affected by the RRi variability. The coefficient estimation follows an iterative identification task based on the

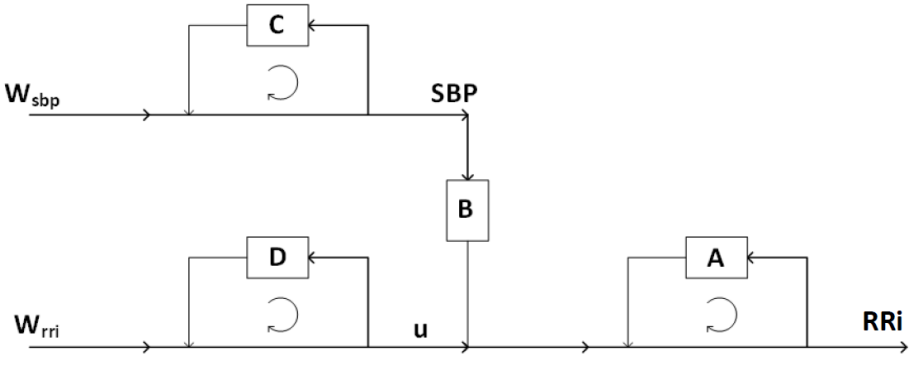

Figure 1. Bivariate autoregressive model with exogenous input (ARXAR model) for the description of the causal effects of systolic blood pressure (SBP) on R-R interval (RRi). In the open loop scheme, RRi values are separately determined by the exogenous input SBP and by SBP-unrelated variations described by the series u [1].

generalized least squares method. The model order p was chosen minimizing the Akaike figure of merit for the bivariate joint process |RRi SBP| [1]. The gain of the RRi – SBP transfer function (G(f)) was estimated directly from the coefficients of the A and B blocks. The value of the gain in the low frequency (LF) band (0.04 – 0.15 Hz) was considered by sampling G(f) on the LF peak of the spectrum of the driving SBP series [26]. It has been shown that there is a good agreement of the causal model with the traditional phenylephrine test to determine baroreceptor responsiveness [1].

STATISTICS

Results are presented as the mean ± SD or percentage, unless stated otherwise. Differences in baseline variables and BR between control subjects and patients were evaluated with the Mann-Whitney test for continuous variables and χ^2 statistics for proportions. Sensitivity and specificity pairs for different BR values distinguishing the groups were determined. These sensitivity and specificity values were used to construct a receiver operating characteristic (ROC) curve, in which the optimal sensitivity and specificity combination is visualized. A p value < 0.05 was considered statistically significant.

RESULTS

Derivation sample

Sample description

Eighteen AD patients (11 women), 19 age-matched healthy control subjects (5 women) and 11 MCI patients (6 women) from RUNMC participated in this study and had the BR measured successfully. Table 1 summarizes the sample characteristics. AD and control subjects did not differ with respect to SBP. However, SBP in MCI patients was higher than in the healthy control subjects ($p < 0.01$).

BR function

BR function was calculated in correspondence with the peak in the power spectra of the SBP between 0.04 and 0.15 Hz (dashed line), the frequency range in which BR modulation of HR occurs. Mean values of the BR gain obtained by the ARXAR model are shown in Figure 2. BR was lower in AD (1.4 ± 0.8 ms/mmHg) compared with control subjects (6.4 ± 2.7 ms/mmHg) ($p < 0.01$). BR in MCI (2.9 ± 0.7 ms/mmHg) differed from both AD ($p < 0.01$) and control subjects ($p<0.01$) and was in between the values for AD and control subjects. The area under

Table 1. Characteristics of derivation and validation sample.

The results are reported as mean ± standard deviation, absolute number or frequencies. MMSE, Mini Mental State Examination; MTA, medial temporal lobe atrophy; HR, heart rate; SBP, systolic blood pressure; ACE inhibitor, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker. * significantly different ($p<0.05$) from the control group of the same sample. † significantly different from the matching group of the derivation sample.

	Derivation sample			**Validation sample**		
	Controls (n = 19)	**MCI** (n = 11)	**AD** (n = 18)	**Controls** (n = 18)	**MCI** (n = 18)	**AD** (n = 16)
Age (years) [mean ± SD]	75 ± 3	74 ± 9	72 ± 6	70 ± 6	70 ± 7	71 ± 8
Sex (male/female)	14 / 5	5 / 6	7 / 11	9 / 9	8 / 10	7 / 9
MMSE (0-30) [mean± SD]	29 ± 5	25 ± 3*	22 ± 5*	29 ± 1	28 ± 1†	21 ± 4*
MTA [mean]	0.6		2*			
Hypertension (n(%))	4 (27)	7 (64)*	6 (33)	9 (50) †	6 (55)	7 (44)
HR (bpm) [mean± SD]	62 ± 8	60 ± 12	71 ± 12	58 ± 6†	60 ± 8	62 ± 7
SBP(mmHg) [mean ± SD]	125 ± 20	152 ± 25*	133 ± 31	136 ± 16	133 ± 17†	133 ± 15
Medication use						
- Beta blocker	2 / 19	4 / 11	5 / 18	0 / 18	3 / 18	0 / 18
- ACE inhibitor	0 / 19	0 / 11	3 / 18	0 / 18	0 / 18	0 / 16
- ARB	0 / 19	0 / 11	1 / 18	0 / 18	0 / 18	0 / 16
- Calcium channel blocker	0 / 19	1 / 11	2 / 18	0 / 18	0 / 18	0 / 16
- Thiazide diuretics	3 / 19	0 / 11	4 / 18	0 / 18	1 / 18	0 / 16
- Vasodilator	0 / 19	1 / 11	1 / 18	0 / 18	0 / 18	0 / 16

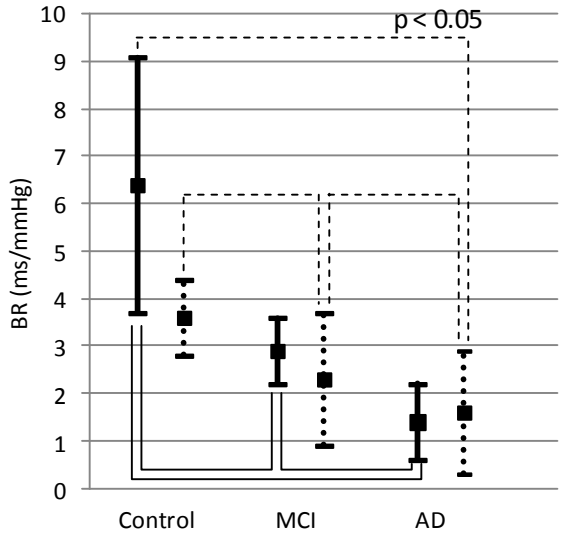

Figure 2. BR function.

BR values in mean ± standard deviation for the three groups (MCI patients, patients with AD and healthy age-matched controls; left graph). The solid lines represent the derivation sample and the dashed lines represent the validation sample. Significant differences are indicated by the connecting lines (dashed for data of the derivation sample,and solid for data of the validation sample).

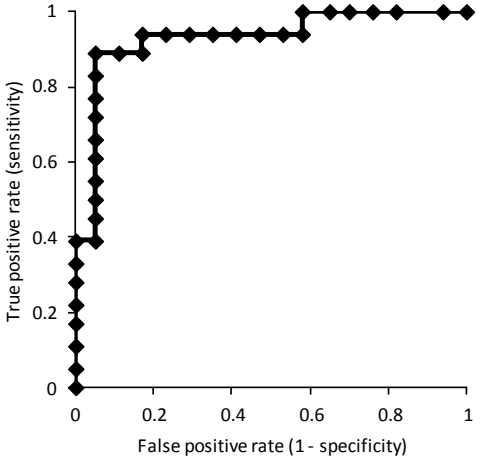

Figure 3. ROC curve.

ROC curve analysis of the predictive value of BR in distinguishing AD patients from healthy controls. The area under the curve equals 0.92; 95 % confidence interval is 0.84-1.00; p-value < 0.01. The square shows the optimal BR cut-off point at 3.2 ms/mmHg resulting in a sensitivity of 89 % and a specificity of 94 % is reached.

Table 2. Performance of the proposed baroreflex cut-off score.

Performance of the proposed baroreflex cut-off score to distinguish between AD and healthy controls, in the validation sample.

Baroreflex value	n of patients (% of total)	AD	Controls	sensitivity	specificity
≤ 3,2 ms/mmHg	18 (51 %)	14	4	93%	78%
> 3,2 ms/mmHg	17 (49 %)	1	14		

the ROC curve for the relationship between AD and healthy elderly was 0.92 (Figure 3). With a threshold (cut-off point) of 3.2 ms/mmHg for AD, sensitivity was 89 % and specificity was 94 %.

Validation sample

Sample description

SBP and RRi data were obtained from 16 AD patients (9 women), 18 age-matched healthy control subjects (9 women), and 18 MCI patients (10 women) from MUMC. Table 1 summarizes their characteristics. In the derivation sample (RUNMC), MCI patients had higher SBP than healthy control subjects. This was not the case in the validation sample from MUMC. There were additional differences between the derivation and validation sample: the MUMC MCI group had lower SBP (133 mmHg) than the RUNMC MCI group (152 mmHg) ($p = 0.02$) and the MUMC control group had a lower HR (58 bpm) compared with the RUNMC control group (62 bpm) ($p = 0.04$).

BR function

BR function was calculated in the same way as for the derivation sample. Mean values for BR gain obtained by the ARXAR model are shown in Figure 2 (dashed lines). Also, BR was lower in AD (1.6 ± 1.3 ms/mmHg) compared with healthy control subjects (3.6 ± 0.8 ms/mmHg) ($p < 0.01$). BR gain in the MCI group (2.3 ± 1.4 ms/mmHg) also was lower than in healthy control subjects ($p < 0.01$) and higher than AD ($p = 0.03$). Application of the BR cut-off value of 3.2 ms/mmHg, as it had been obtained in the derivation sample, yielded a sensitivity of 93% and a specificity of 78% for differentiating AD from healthy control subjects in this validation dataset (Table 2).

Cholinesterase inhibitors

In 18 AD patients (11 women) in the derivation sample, SBP and RRi data were obtained before and after treatment with cholinesterase inhibitors. After treatment with cholinesterase inhibitors, BR increased from 1.4 ± 0.8 ms/mmHg to 2.4 ± 0.9 ms/mmHg ($p < 0.01$). In the validation sample, no before and after cholinesterase inhibitor treatment comparisons were available, however in this sample, BR was higher in the 10 patients who were treated with cholinesterase inhibitors (2.0 ± 1.5 ms/mmHg) compared with the 6 patients who were not treated (0.89 ± 0.4 ms/mmHg) ($p = 0.02$). Cholinesterase inhibitor-treated patients in the derivation sample had a slightly higher BR than those in the validation sample ($p = 0.03$).

DISCUSSION

The main finding of our study is that the BR function is lowered in patients with AD compared with age-matched control subjects. Our results are in agreement with the depressed BR sensitivity observed in 24 patients with AD [27]. Our study adds to these observations by the addition of a group of patients with MCI, reflecting early-stage AD, by reproducing our findings in an independent sample and by investigating the effect of cholinesterase inhibitors on BR. Furthermore, a more precise BR quantification method was used. The traditional sequence analysis used in the study of Szili-Török et al. [27] assumes, but not tests, that the whole RRi variability is generated by SBP changes. The method used in our study takes this causal dependency into account by dividing the RRi variability in SBP-related and SBP-unrelated parts.

The difference in BR function was so prominent that it was possible to distinguish patients with AD from control subjects by their BR function. The discriminatory value of BR was so strong that it achieved a performance that compares with or even exceeds that of current diagnostic biomarkers, such as cerebrospinal fluid analysis of amyloid β42. Indeed, a cut-off value for BR gain of 3.2 ms/mmHg reached a sensitivity of 89 % and a specificity of 94 % to discriminate AD from control subjects in the derivation sample. For comparison, cerebrospinal fluid analysis of amyloid β42 has an ROC of 0.9, with a sensitivity of 93 % and a specificity of 87 % to discriminate AD from control subjects [28]. This discriminatory cut-off value for BR performed equally well in the independent validation sample with a sensitivity of 93 % and a specificity of 78 % for differentiating AD from healthy control subjects. Additionally, MCI patients had a BR value that was intermediate between control subjects

and AD. A strength of our study is that these results were also confirmed in the independent validation sample.

168 These findings underscore the importance of exploring the underlying mechanism that explains this association between impaired BR functioning and AD. They raise the question whether AD pathology results in impaired BR functioning or whether impaired BR functioning contributes to (acceleration of) AD pathology.

Possible mechanism and consequence of the decreased BR in AD

The BR is the major feedback control system for BP. Primary afferent fibres from arterial baroreceptors —in the carotid sinus and aortic arch—send information to the nucleus tractus solitarius in the medulla oblongata [29]. From here, information is sent via interneurons to the hypothalamus or to higher regions in the brainstem and forebrain [30], including the insular cortex [31]. The insular cortex is a recognized site of autonomic cardiovascular control and BR-mediated autonomic cardiovascular function [32]. In 1998, Braak and Braak [33] demonstrated a hierarchical progression of AD pathology that includes the insular cortex in an early stage. This insular involvement might lead to changes in cardiovascular and autonomic control, and might consequently affect the BR [32, 34].

Further circumstantial evidence is found in the effects of exercise. Exercise improves cardiovascular function and BR [35]. Exercise has also been shown to lower the risk of AD [36]. Although direct associations between exercise, BR and AD risk have not been investigated, and BR could be no more than a marker of exercise, it can be hypothesized that BR function is part of the causal pathway that links exercise to reduced AD risk, or that augmented BR is a marker of beneficial effects of exercise on autonomic brain centres.

Here, we have also shown that treatment with cholinesterase inhibitors augmented BR function in AD. This is in line with recent findings that atropine, in a dose which augments vagal tone, increases cardiovagal BR gain in older subjects [37]. Our findings cannot be explained by age-related effects only [38] because we compared AD and MCI patients with age-matched healthy control subjects. Cholinesterase inhibitors partly compensate for the cholinergic deficit in AD. The cholinergic system has a pivotal role in learning and memory [39]. However, the augmented BR function suggests that cholinesterase inhibitors might slow clinical disease progression not only through direct cognitive effects but also through their influence on cardiovascular factors such as BP regulation.

Our findings might extend to preclinical and early clinical AD, for example, patients with MCI. Patients with MCI have a high risk of progression to dementia, particularly of the Alzheimer type [21, 40]. There is a greater orthostatic fall in SBP in MCI and Alzheimer patients than in control subjects [23, 24] and the prevalence of orthostatic hypotension in MCI patients lies between control subjects and AD patients [41]. This observation parallels the BR values in control subjects, MCI, and AD found in our study and is in line with an earlier study which showed an association between blunted BR functioning and increased risk of orthostatic hypotension [42].

Despite the fact that several studies have shown an association between AD and autonomic instability, such as increased pupillary dilatation [43], diminished HR variability [44] and orthostasis, the significance of the abnormalities in BR among AD patients is not yet fully known. However, impaired BR in other common dementia subtypes, such as Parkinson disease and Lewy body disease, is held partly responsible for the higher prevalence of orthostatic hypotension [24] and increased cardiovascular mortality. These patients also exhibit a significant loss of cholinergic forebrain neurons [45].

BR functioning as a diagnostic tool

In the perspective of new therapeutic options to slow the progression of dementia, an early diagnosis of AD is of utmost importance because all treatment strategies are more effective in the earlier phases of the disease [46]. In this study, a rapid, non-invasive and inexpensive procedure without need for active patient cooperation was used to estimate BR function from ECG and BP recordings, obtained when the patient quietly sits or lies down. Our results show that in patients with AD, a lowered BR could be a sensitive and specific marker of the disease. A strength of the study is the consistent decrease in BR found over the different groups (from healthy subjects to MCI patients to AD patients). This result points toward a disease-specific involvement of the BR. However, although we have shown that BR discriminates between AD and control subjects, further evidence must be obtained for its discriminatory value against important differential diagnoses in memory clinics (i.e., other causes of dementia and depression).

Limitations

Some methodological issues need to be considered. First of all, the diagnosis of AD and MCI in our study remains a clinical one. There was no pathologic confirmation of the underlying

disease. However, previous studies have shown that a clinical diagnosis is only inaccurate, compared with pathologic diagnosis, in approximately 11 % of mild cases [47]. The patients with MCI and AD were diagnosed by a multidisciplinary memory clinic team consisting of several geriatricians, neuropsychologists, occupational therapists, and speech therapists. Lewy body dementia and vascular dementia were excluded based on diagnostic criteria for these conditions, using information obtained by means of history, clinical examination, laboratory tests, and additional, mostly radiologic, investigations. In a population referred to a memory clinic (as in this study), depending on criteria, a high percentage of MCI patients will develop AD [48, 49]. Still, in some patients MCI remains stable and never progresses to AD. Therefore the assumption that abnormal BR function serves as early detection of the Alzheimer disease process remains hypothetical. To overcome this limitation, larger scale studies with follow-up of MCI and AD patients are needed, with observations of BR during the different stages of progression of AD. Maybe a combination with other easy to determine parameters, in particular the cerebrovascular resistance index [18], could enhance discrimination of patients at risk for developing AD.

Second, it might be argued that results should have been corrected for differences in use of medication, because we included patients with comorbidities and medication. There is some information about whether commonly used antihypertensive agents, such as metoprolol and enalapril, can augment the cardiac autonomic function in hypertensive patients. Keeley et al. showed that β-blockers can directly improve cardiovascular autonomic regulation in normotensive and hypertensive patients [50]. Further, Mancia et al. showed an increase in BR function when the circulating levels of angiotensin II and aldosterone were reduced by angiotensin-converting enzyme inhibitors [51]. Because antihypertensive drugs mostly show an increase of the BR function, we argue that the difference in BR function between AD patients and healthy elderly would have been even greater when the AD patients had used no antihypertensive agents.

Third, some differences exist in population and protocol characteristics between the derivation and validation sample. In the validation sample the control subjects had a higher SBP (136 mmHg compared with 125 mmHg) than in the derivation sample. Furthermore, in the derivation sample BP and HR were obtained in the sitting position, in the validation sample BP and HR were obtained supine. These differences might explain the difference in BR between the 2 control groups (6.4 ms/mmHg in the derivation sample versus 3.6 ms/mmHg in the validation sample). Bristow et al. showed an inverse relationship between resting mean BP and BR, with reduced BR sensitivity in hypertension [52]. This might explain the observed

lower BR in the derivation sample where the SBP was higher. However, literature yields discordant results about the influence of posture on the BR results [53, 54].

Finally, a limitation of the study is the cross-sectional design. This design allows us to establish the relationship between BR and AD, but we are unable to establish whether the impaired functioning of the BR is the effect or the cause of neurodegeneration.

CONCLUSIONS

This study shows a strong association between AD and diminished BR function. A possible link is the cholinergic system which is involved in both BR and AD. Cholinesterase inhibitors increase BR function in AD. Furthermore, MCI patients have an intermediate BR function between the normal and the AD subjects. Considering this, it is possible that early in AD besides cognition also BP regulation is affected. Further research on the relationship between BR and AD might provide valuable insights into the pathophysiology and for the diagnosis and treatment of AD.

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Chapter 8.

Very-low-frequency oscillations of cerebral haemodynamics and blood pressure are affected by aging and cognitive load

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ABSTRACT

Spontaneous slow oscillations occur in cerebral haemodynamics and blood pressure (BP), and may reflect neurogenic, metabolic or myogenic control of the cerebral vasculature. Aging is accompanied by a degeneration of the vascular system, which may have consequences for regional cerebral blood flow and cognitive performance. This degeneration may be reflected in a reduction of spontaneous slow oscillations of cerebral haemodynamics and BP. Therefore, we aimed to establish the dependency of slow oscillations of cerebral haemodynamics and BP on the factors age and cognitive load, by using functional Near-Infrared Spectroscopy (fNIRS). Fourteen healthy young (23 - 32 years) and 14 healthy older adults (64 - 78 years) performed a verbal n-back working-memory task. Oxygenated and deoxygenated haemoglobin concentration changes were registered by two fNIRS channels located over left and right prefrontal cortex. BP was measured in the finger by photoplethysmography. We found that very-low-frequency oscillations (0.02 - 0.07 Hz) and low-frequency oscillations (0.07 - 0.2 Hz) of cerebral haemodynamics and BP were reduced in the older adults compared to the young during task performance. In young adults, very-low-frequency oscillations of cerebral haemodynamics and BP reduced with increased cognitive load. Cognitive load did not affect low-frequency oscillations of the cerebral haemodynamics and BP. Transfer function analysis indicated that the relationship between BP and cerebral haemodynamic oscillations does not change under influence of age and cognitive load. Our results suggest aging-related changes in the microvasculature such as declined spontaneous activity in microvascular smooth muscle cells and vessel stiffness. Moreover, our results indicate that in addition to local vasoregulatory processes, systemic processes also influence cerebral haemodynamic signals. It is therefore crucial to take the factors age and BP into consideration for the analysis and interpretation of haemodynamic neuroimaging data.

INTRODUCTION

Neuroimaging with functional Near-Infrared Spectroscopy (fNIRS) and fMRI has registered the occurrence of spontaneous slow oscillations of cerebral haemodynamics [1]. The driving force for these oscillations may vary between neurogenic, metabolic and myogenic control of the cerebral vasculature [2-5]. Different physiological origins for slow oscillations of cerebral haemodynamics are suggested in the literature and may be summarized as follows: 1) spontaneous slow changes in cerebrovascular tone (vasomotion), 2) changes in systemic haemodynamics (blood pressure (BP)) reflected in cerebral haemodynamics and 3) slow oscillations in neuronal activation, related to functional network connectivity. Slow oscillations are further characterized by their frequencies. Very-low-frequency oscillations (VLFOs) occur at approximately 0.04 Hz and low-frequency oscillations (LFOs) are centred around 0.1 Hz [6]. These slow oscillations can thus be differentiated from high-frequency oscillations (HFOs) that are known to be of respiratory origin, around 0.2 - 0.3 Hz, and the heartbeat cycles that occur at approximately 1 Hz [7].

Slow oscillations of cerebral haemodynamics are modulated by functional stimulation. Obrig et al. (2000) established using fNIRS that functional activation affects slow oscillations of cerebral haemodynamics in the visual cortex in young adults [6]. In comparison to rest, visual checkerboard stimulation reduced VLFOs of oxygenated haemoglobin ([O_2Hb]), centred around 0.04 Hz. No significant effects of functional activation were found for VLFOs of deoxygenated haemoglobin ([HHb]) or for LFOs centred around 0.10 Hz. For functional activation research it is relevant to know how slow oscillations are affected not only by functional stimulation versus rest, but also by cognitive load. To date, however, it is unclear if and how cognitive load influences these oscillations. Furthermore, because systemic BP oscillations have been investigated together with cerebral haemodynamics only in a relatively small number of studies, it remains insufficiently known to what extent the effects of cognitive load on cerebral oscillations may be mediated by effects on these systemic oscillations.

In addition to functional activation, slow oscillations may be affected by aging. Schroeter et al. showed with fNIRS that LFOs (0.07 - 0.11 Hz) of [O_2Hb] and [HHb] in the cerebral microvasculature strongly declined with aging during both rest and visual checkerboard stimulation [8]. VLFOs (0.01 - 0.05 Hz) were not affected by age, but functional stimulation increased VLFOs of [O_2Hb] and [HHb] in young adults and increased VLFOs of [HHb] in older adults. These results might indicate a decline in spontaneous activity in microvascular smooth

muscle cells in conjunction with an increase in vessel stiffness in the elderly. Aging is further accompanied by a degradation of the cerebrovascular system encompassing changes in resting cerebral blood flow, vascular reactivity and vascular ultrastructure [9]. For example,
180 changes in the ultrastructural integrity of the cerebral vasculature result in a decrease in the elasticity and compliancy of affected vessels, including capillaries, larger arterioles and cerebral arteries [10]. Accordingly, the diversity of aging-related vascular pathological changes may have a large influence on the cerebral haemodynamic oscillations, and hence on the interpretation of haemodynamic neuroimaging data [11].

Recently, spontaneous slow oscillations of the fMRI BOLD-signal have gained much interest. Specifically, it has been suggested that these oscillations reflect spontaneous neuronal activity and that they may play a role in functional connectivity between different brain regions. Accordingly, several studies have focused on inter-regional correlations in slow BOLD oscillations during resting-state and task performance [12]. Sambataro et al. investigated slow oscillations (0.03 - 0.08 Hz) of the fMRI BOLD-signal during performance of the n-back working-memory task [13]. In older adults oscillations were reduced in power in posterior regions of the default mode network in comparison to young adults. With increasing cognitive load (1-back and 2-back versus 0-back), power decreased in both groups, but the power attenuation was smaller in older adults. The authors concluded that older adults show decreased functional connectivity and a decreased ability to suppress slow oscillations of the default mode network. Systemic oscillations were not measured in that study however.

It is relevant to know how the amplitude of the haemodynamic oscillations is affected by different cognitive loads, since aging-related changes in these oscillations are likely to reflect aging-related changes in neurogenic, metabolic or myogenic regulation of microvascular blood flow. This knowledge will contribute to improved analysis and interpretation of haemodynamic neuroimaging data. Therefore, the first aim of this study was to examine interaction effects of age and cognitive load on oscillations of cerebral haemodynamics by using functional fNIRS, a non-invasive neuroimaging technique which is particularly sensitive to the microvasculature.

When analysing task-related changes in the regional cerebrovascular response, the systemic response is often neglected or assumed to be unchanged. However, Tachtsidis et al. found significant task-related changes in both regional cerebral haemodynamic and systemic signals during functional activation of the frontal cortex. In some participants, these changes were highly correlated [14]. These results suggest a centrally mediated mechanism influencing both

the cerebrovascular and cardiovascular systems. Therefore, the second aim of our study was to examine the impact of age and cognitive activation on BP oscillations. We performed transfer function analysis to gain more insight into the relationship between the task-induced BP oscillations and cerebral haemodynamic oscillations.

Taken together, we hypothesized that not only aging, but also cognitive load may affect slow oscillations of cerebral haemodynamics. To enhance our understanding of the origins of these oscillations, we recorded both cerebral and systemic BP oscillations and investigated their possible relationship using transfer function analysis.

MATERIALS AND METHODS

Participants

Fourteen healthy young adults (8 female, mean age = 26.4 ± 3.0 years, range 23 - 32) and 14 healthy older adults (10 female, mean age = 70.3 ± 4.7 years, range 64 - 78) participated in this study. Educational level slightly differed between the young (M = 16.7 ± 2.8 years, range 10.5 - 18.0) and older adults (M = 12.6 ± 3.2 years, range 9.0 - 18.0) (Mann-Whitney U = 33.00, p = .002). All participants completed secondary school or higher. None of the older adults experienced subjective memory problems, all were living independently and all had unimpaired overall cognitive function as assessed with the Mini Mental State Examination ([15]; mean score = 29.1 ± 0.9, range 27 - 30). All participants were right-handed and had normal or corrected-to-normal vision. None of the participants had a history of neurological or psychiatric disease, or used psychopharmacological drugs. Four older adults used antihypertensive medication. All participants refrained from alcohol, caffeine and nicotine from at least 3 h before the experimental session. The study was approved by the local medical ethics committee and all participants gave written informed consent.

Experimental procedure

The experimental procedure utilized in the present study and the accompanying behavioural results have previously been described in detail [16]. Participants performed two versions of a verbal n-back task; after the 0-back task (control condition) the 2-back task (high working-memory load condition) was realized. This paradigm has reliably and validly been employed in establishing cerebral activity patterns in the prefrontal cortex in relation to increasing working-memory load in fMRI research [17, 18]. The 2-back task places a large

demand on a number of key processes within working memory. The 0-back task has regularly been used as control condition to measure attention and alertness without working-memory load. Since the aim of our study was to specifically investigate the effects of working-memory load, and cognitive processes are uncontrolled during resting periods, we decided to use 0-back as control situation instead of rest.

Prior to both conditions, participants practiced the task for 1 min and received feedback about their performance. Both conditions were preceded by a baseline period of 1 min, during which a black fixation cross was displayed at the centre of the 15 inch screen. Both conditions consisted of 60 trials, 17 of which were target trials. In each trial, a letter that was randomly selected from a set of 20 consonants was presented in black on a light gray background with a presentation time of 500 ms. Interstimulus interval was 3000 ms. During each trial, participants indicated whether the stimulus was a target by pressing the button under the right index finger, or a non-target by pressing the button under the right middle finger (PST Serial Response Box, Psychology Software Tools Inc., PA, USA). In the 0-back condition, the letter "X" was defined as target. In the 2-back condition, the target was any letter that was identical to the letter presented two trials before, while the letter "X" was no longer shown.

Data acquisition

We used a continuous-wave NIRS device (Oxymon Mk III, Artinis Medical System, The Netherlands), using light of three wavelengths (765, 857, 859 nm), to monitor concentration changes in cortical oxygenated haemoglobin ([O_2Hb]) and deoxygenated haemoglobin ([HHb]) with high temporal resolution. The principle behind fNIRS is that near-infrared light penetrates the skull and brain and is absorbed by the chromophores O_2Hb and HHb, which have different absorption spectra. Assuming constant scattering [19] and by using the modified Lambert-Beer Law, it is possible to calculate the concentration changes of these chromophores in the penetrated brain tissue based on changes in the detected light intensity. Both increases in [O_2Hb] and decreases in [HHb] are indicators of cortical activation. Concentration changes in total haemoglobin ([tHb]), defined as the sum of changes in [O_2Hb] and [HHb], represent an indicator of alterations in total blood volume.

In the present study, two pairs of optodes were bilaterally attached to the forehead and were tightly fixed in a customized headband (Spencer technologies, Seattle, WA). The detection optodes were placed 25 - 30 mm above the midpoint of the eyebrow, at approximately FP1

and FP2 according to the international 10 - 20 electrode system. The emission optodes were laterally placed at approximately F7 and F8. The emitter-detector spacing was 50 mm to minimize contamination from the extra-cerebral circulation and maximize signal intensity [20,21]. The differential pathlength factor (DPF), which accounts for the increased distance travelled by light due to scattering, is age-dependent [22]. For the young adults, DPF was calculated by the formula $4.99 + 0.067 \times Age^{0.814}$. At present however, no data are available on the actual variation of DPF in adults aged above 50 years. Therefore, the DPF was set to 6.61, corresponding to age 50, in the older adults [22, 23].

BP was measured simultaneously using a photoplethysmography cuff on the index or middle finger of the left hand of the participant (Finometer, FinaPres Medical Systems, the Netherlands). A three-lead ECG was recorded for measurement of the R-R interval.

Data processing

BP, ECG, [O_2Hb], [HHb] and [tHb] were simultaneously recorded with a sample frequency of 125 Hz. Analyses were performed with MATLAB (MathWorks, MA, USA). Samples obtained from the left and right fNIRS channels were averaged for each time point. From the BP recordings, mean beat-to-beat blood pressures were extracted.

Mean baseline values of [O_2Hb] and BP were calculated over the last 20 s of the pre-task baseline period. To establish the haemodynamic and systemic responses to cognitive performance, mean values of [O_2Hb] and BP were calculated over 180 s of the 0-back and 2-back task period, respectively. The first three trials (all non-targets) of both conditions were excluded from data analyses.

Beat-to-beat changes in mean BP, [O_2Hb], [HHb] and [tHb] were aligned with the time of the R wave peak of the ECG. The time series were cubically interpolated at 1 Hz to obtain uniformly spaced time series for spectral and transfer function analysis. The signals were linearly detrended and were filtered using a Butterworth high-pass filter with a cut-off frequency of 0.02 Hz. Spectral analysis was performed on 180-second data segments of BP, [O_2Hb], [HHb] and [tHb] of both the 0-back and 2-back task. Spectral estimation was based on the Welch algorithm [24]. Spectral estimates were determined as the average of 50-second windows, overlapping by half. In this way, each data segment contains at least one full period of oscillations at the lowest frequency (0.02 Hz). The following frequency ranges were chosen for spectral analysis: very-low-frequency (VLF; 0.02 - 0.07 Hz), low-frequency (LF; 0.07 - 0.2

Hz) and high-frequency (HF; 0.2 - 0.35 Hz).

The relationship between oscillations of BP and [O_2Hb] was studied by means of transfer function analysis. The parameters gain, phase and coherence were estimated using the cross-spectral method which has been described in detail previously [25-27]. Fast Fourier transforms were implemented with each Hamming-windowed segment and averaged to quantify the transfer function.

It should be noted that the downstream [O_2Hb] oscillations are related to BP oscillations, and are also influenced by the relationship between oscillations of BP and upstream cerebral blood flow in conduit and resistance vessels, which is subject to continuous cerebral autoregulatory action. Thus, in short, the parameter gain quantifies how the amplitudes of the oscillations in BP are transmitted to the oscillations of [O_2Hb]; a lower gain implies that these oscillations are reduced by efficient dynamic cerebral autoregulation, or might reflect enhanced metabolic reserve or enhanced diffusion of oxygen [20]. The phase shift describes the time relationship between oscillations of BP and [O_2Hb] and may reflect cerebral autoregulatory action and circulatory transit times [28]. Coherence indicates the linearity of the relation between BP and [O_2Hb] oscillations. Coherence approaching unity suggest a linear relationship, while coherence approaching zero suggest no relationship between the signals, severe extraneous noise, or a non-linear relationship. Data with coherence ≤ 0.1 for VLF were excluded from analysis; data with coherence $> 0.1 < 0.4$ in VLF were not rejected if corresponding coherence in LF was > 0.4. This threshold of 0.1 was chosen because a higher threshold for VLF would lead to a biased rejection of estimates of gain and phase. With active and intact cerebral autoregulation coherence values will be by definition low in the VLF range where cerebral autoregulation is most active [29].

Accordingly, data from 12 young and 13 older adults were included for transfer function analysis in the VLF range, and data from 14 young and 12 older adults for analysis in the LF range. Because the coherence between BP and [O_2Hb] oscillations in the HF range approached zero in almost half of the participants, no analyses were performed in this frequency range. Transfer function analysis was not applied to the relationship between BP and [HHb], since oscillations of [HHb] are too weak for a reliable calculation of transfer function estimates.

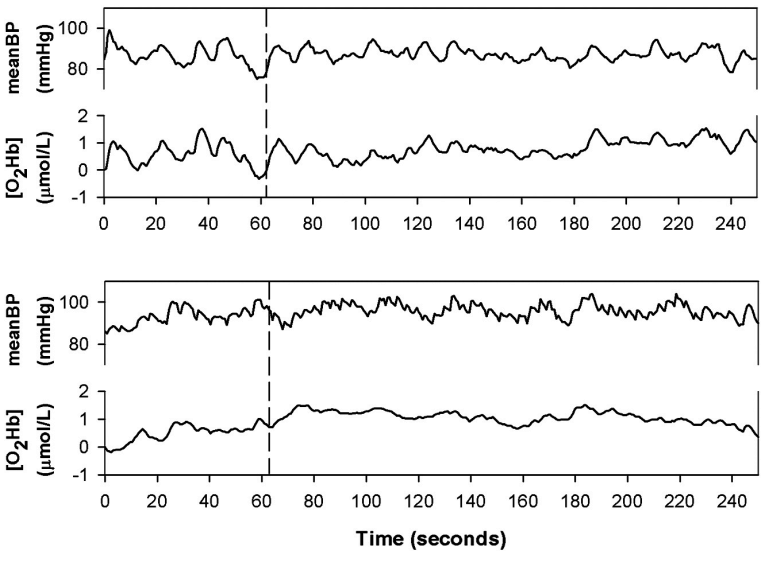

Figure 1. Beat-to-beat time series.

Mean blood pressure (BP) and oxygenated haemoglobin [O_2Hb] from a representative young participant (upper panel) and an older participant (lower panel) during baseline and 0-back performance. The dashed line marks the start of the task. Note that in the lower panel high-frequency oscillations of blood pressure are clearly visible.

Table 1. Spectral power peaks.

Averages (mean ± standard deviation) of spectral power peaks in the different frequency bands in young and older adults during 0-back and 2-back performances. Note that subjects were left out of the calculation of the average spectral power peak if they did not show a clear peak in that particular frequency band. No clear peaks were found for HFOs of [O2Hb]. BP = blood pressure; [O2Hb] = concentration changes in oxygenated haemoglobin; VLF = very-low-frequency range (0.02 - 0.07 Hz); LF = low-frequency range (0.07 - 0.2 Hz); HF = high-frequency range (0.2 - 0.35 Hz).

	0-back **Frequency (Hz)**				2-back **Frequency (Hz)**			
	Young adults		**Older adults**		**Young adults**		**Older adults**	
BP VLF	0.044	± 0.009	0.045	± 0.016	0.039	± 0.011	0.045	± 0.012
BP LF	0.097	± 0.021	0.102	± 0.023	0.101	± 0.026	0.099	± 0.014
BP HF	0.282	± 0.008	0.278	± 0.01	0.278	± 0.031	0.27	± 0.03
[O_2Hb] VLF	0.041	± 0.011	0.044	± 0.017	0.04	± 0.011	0.039	± 0.014
[O_2Hb] LF	0.096	± 0.012	0.088	± 0.011	0.094	± 0.015	0.097	± 0.023

STATISTICAL ANALYSIS

Statistical analysis was performed using PASW Statistics software version 18.0 (SPSS Inc.,
186 Chicago, IL, USA). Statistical significance was set at an alpha of .05. Data are presented as mean ± SD. Shapiro-Wilk tests indicated that the power spectral data were not normally distributed. Accordingly, the power spectral data were log transformed before statistical testing. For the transfer function estimates, the assumption of normality was met (Shapiro-Wilk). Comparisons between the young and older adult groups, and between the high working-memory load condition and control condition were made using repeated-measures ANOVA. Significant main and interaction effects were further analysed by means of planned contrasts.

RESULTS

Representative oscillations of BP and [O_2Hb] during task performance are presented in Figure 1. Table 1 shows the averaged spectral power peaks in the VLF, LF and HF range in young and older adults during 0-back and 2-back performances.

Cerebral haemodynamic measurements

During 0-back performance, mean [O_2Hb] increased in comparison to baseline 0.32 ± 0.25 µmol/L in older adults ($F(1,13) = 23.13$, $p < .001$). No significant change of [O_2Hb] was detected during 0-back performance in young adults (0.19 ± 0.46 µmol/L; ($F(1,13) = 2.32$, $p = .151$). Mean [O_2Hb] changes from baseline did not differ between groups ($F(1,26) = 0.94$, $p = .341$).

During 2-back performance, mean [O_2Hb] increased in comparison to baseline 0.34 ± 0.43 µmol/L in young adults ($F(1,13) = 8.75$, $p = .011$)) and 0.61 ± 0.23 µmol/L in older adults ($F(1,13) = 99.61$, $p < .001$). Mean [O_2Hb] changes from baseline marginally differed between groups ($F(1,26) = 4.19$, trend: $p = .051$).

Spectral analysis of cerebral oscillations

Figure 2 demonstrates that the magnitude of VLFOs in [O_2Hb] and [tHb] is influenced by both age and working-memory load. VLFOs of [O_2Hb] ($F(1,26) = 9.70$, $p = .004$) and [tHb] ($(1,26) = 13.18$, $p = .001$) were stronger in young adults compared to older adults

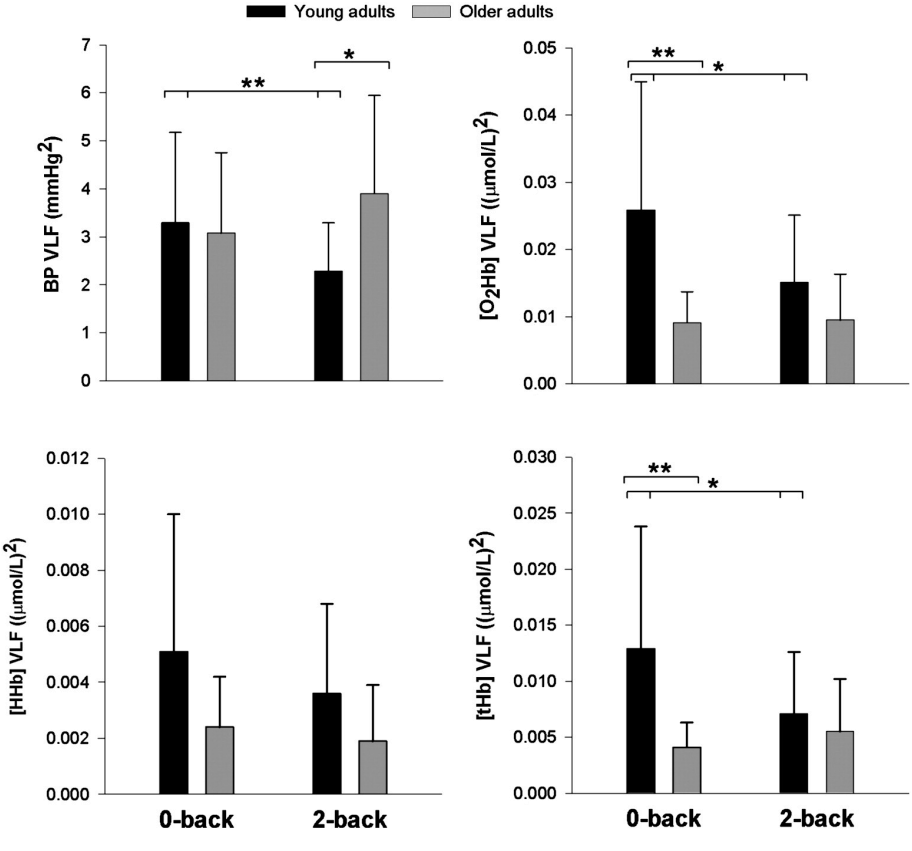

Figure 2. Spectral power.

Spectral power of very-low-frequency oscillations in blood pressure (BP) and cortical haemoglobin in young and older adults during 0-back and 2-back performances. Bar graphs show the untransformed power spectral density values (mean ± standard deviation) for oscillations in BP, oxygenated, deoxygenated and total haemoglobin ([O_2Hb]; [HHb]; [tHb]) in the very-low-frequency range (VLF = 0.02 - 0.07 Hz). Note however, that statistical analysis has been performed on log transformed data. *$p < .05$, **$p < .005$.

Table 2. Untransformed power spectral density values of low- and high-frequency oscillations of blood pressure and cortical haemoglobin in young and older adults during 0-back and 2-back performances. BP = blood pressure; [O2Hb], [HHb], [tHb] = concentration changes in oxygenated, deoxygenated and total haemoglobin, respectively; LF = low-frequency range (0.07 - 0.2 Hz); HF = high-frequency range (0.2 - 0.35 Hz). *p < .05, **p < .005.

	0-back		2-back	
	Young adults	Older adults	Young adults	Older adults
BP LF ($mmHg^2$)	4.484 ± 2.9861	1.6832 ± 1.4272**	4.5353 ± 3.3221	1.6222 ± 1.0363**
BP HF ($mmHg^2$)	0.44 ± 0.3086	0.6318 ± 0.61	0.4464 ± 0.3384	0.5888 ± 0.6532
[O_2Hb] LF (($\mu mol/L)^2$)	0.0175 ± 0.0135	0.0037 ± 0.0038**	0.0155 ± 0.011	0.0036 ± 0.0022**
[O_2Hb] HF (($\mu mol/L)^2$)	0.0007 ± 0.0003	0.0002 ± 0.0002**	0.0007 ± 0.0006	0.0003 ± 0.0004*
[HHb] LF (($\mu mol/L)^2$)	0.0038 ± 0.0048	0.0007 ± 0.0007**	0.0026 ± 0.0027	0.0006 ± 0.0005**
[HHb] HF (($\mu mol/L)^2$)	0.0002 ± 0.0001	0.0001 ± 0.0000**	0.0002 ± 0.0002	0.0001 ± 0.0001*
[tHb] LF (($\mu mol/L)^2$)	0.0084 ± 0.0058	0.0019 ± 0.0016**	0.0081 ± 0.0066	0.0025 ± 0.0018**
[tHb] HF (($\mu mol/L)^2$)	0.0007 ± 0.0005	0.0002 ± 0.0002*	0.0007 ± 0.0006	0.0004 ± 0.0008

during the control condition. Power of VLFOs of [O_2Hb] was reduced by 23.4% with increased working-memory load in young adults ($F(1,13) = 8.07$, $p = .014$) and became similar to the magnitude of VLFOs in older adults ($F(1,26) = 4.09$, $p = .053$). Power of VLFOs of [tHb] declined 22.5 % with increased working-memory load in young adults ($F(1,13) = 5.30$, $p = .039$) and became similar to the magnitude in older adults ($F(1,26) = 1.67$, $p = .208$). VLFOs of [HHb] were not influenced by cognitive load or age.

The spectral power of LFOs and HFOs of [O_2Hb], [HHb] and [tHb] was not influenced by differences in cognitive load, but overall declined with age (Table 2).

Systemic measurements

Mean BP was higher in older adults than in young adults in all conditions. In comparison to baseline measurements, BP rose slightly under high working-memory load in young (4.3 ± 4.4 mmHg, $F(1,13) = 13.39$, $p = .003$) and older adults (5.9 ± 5.0 mmHg, $F(1,13) = 19.51$, $p = .001$), but the increase did not differ between groups ($F(1,26) = 0.83$, $p = .371$).

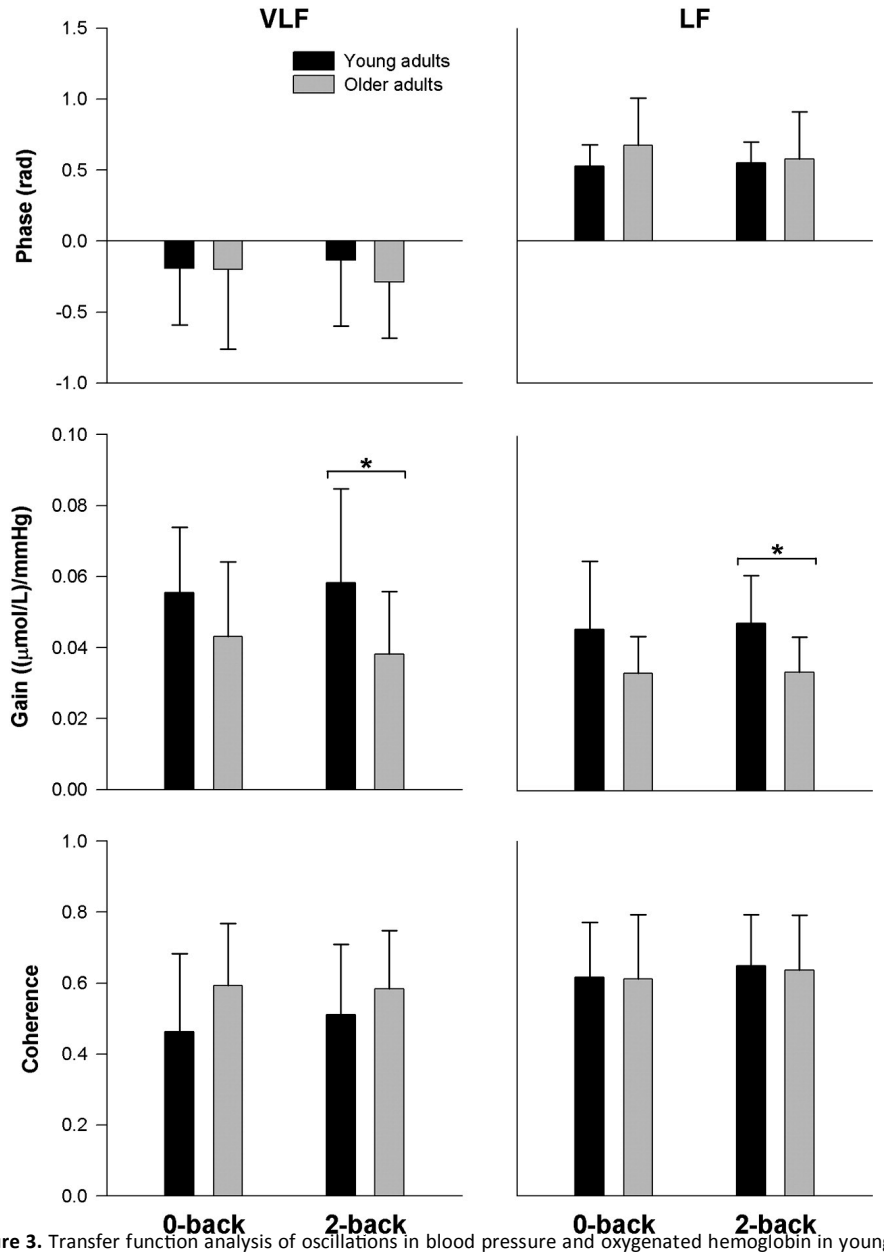

Figure 3. Transfer function analysis of oscillations in blood pressure and oxygenated hemoglobin in young and older adults during 0-back and 2-back performances. Bar graphs show the values (mean ± standard deviation) of phase (top panels), gain (middle panels), coherence (bottom panels) for oscillations in the very-low-frequency range (VLF = 0.02 - 0.07 Hz)and low-frequency range (LF = 0.07 - 0.2 Hz). *p < .05.

Spectral analysis of systemic oscillations

In the control condition, VLFOs of BP were equal in power for young and older adults. However, high working-memory load resulted in a power decline of 24.0 % in young adults ($F(1,13) = 13.78$, $p = .003$), while in older adults the VLFOs did not change ($F(1,13) = 2.26$, $p = .156$). The spectral power of LFOs of BP was not influenced by cognitive load, but declined with age. HFOs of BP were not affected by either working-memory load or age (Table 2).

Relationship between cerebral and systemic oscillations

Figure 3 gives an overview of the transfer function parameters phase, gain and coherence for oscillations of BP and [O_2Hb] in the VLF (left panel) and LF range (right panel) during 0-back and 2-back performances. No within- or between-group differences were found for transfer function parameters phase and coherence. In the VLF range, phase shift was on average slightly negative, but varied largely across participants; BP oscillations followed [O_2Hb] oscillations in some participants, while they led [O_2Hb] oscillations in others. In the LF range, BP oscillations led [O_2Hb] oscillations in all participants. Coherence values between BP and [O_2Hb] oscillations were as follows: in young adults 0.46 ± 0.22 (0-back) and 0.51 ± 0.20 (2-back) in the VLF range, and 0.62 ± 0.16 (0-back) and 0.65 ± 0.14 (2-back) in the LF range; in older adults 0.59 ± 0.18 (0-back) and 0.58 ± 0.16 (2-back) in the VLF range, and 0.61 ± 0.18 (0-back) and 0.64 ± 0.15 (2-back) in the LF range (Figure 3).

For the parameter gain, we found an age effect in the VLF range ($F(1,23) = 5.35$, $p = .030$) and LF range ($F(1,24) = 7.24$, $p = .013$). Further testing revealed this effect was only present for the 2-back task (VLF range ($F(1,23) = 5.15$, $p = .033$); LF range ($F(1,24) = 8.75$, $p = .007$)). However, no significant effects of working-memory load or interaction effects between working-memory load and age were present, which indicates that possible age effects on gain did not significantly differ between the different levels of working-memory load. Therefore, we conclude that there is no clear effect of age on gain.

Taken together, the results of transfer function analysis show a clear relationship between systemic and cerebral oscillations. This relationship displays the known properties of cerebral autoregulation. We found no overall effects of age and working-memory load on the relationship between BP and [O_2Hb] oscillations.

DISCUSSION

The aim of our fNIRS study was to examine interaction effects of age and cognitive load on VLFOs (0.02 - 0.07 Hz), LFOs (0.07 - 0.2 Hz) and HFOs (0.2 - 0.35 Hz) of cerebral haemodynamics and BP. In young adults, increased working-memory load resulted in a reduction of VLFOs of cerebral haemodynamics and BP. Moreover, our study shows that VLFOs, LFOs and HFOs of cerebral haemodynamics declined with aging. BP oscillations in the LF range also declined with aging. Transfer function analysis identified a relationship between BP and [O_2Hb] oscillations. Furthermore, it demonstrated that this relationship did not change under influence of age or cognitive load.

The present study is the first to utilize fNIRS to investigate the effects of cognitive load on slow oscillations in cerebral haemodynamics in both young and older adults. Our results are in agreement with the studies of Obrig et al. [6] and Schroeter et al. [8]. From these studies and the current study, it can be concluded that VLFOs are affected by functional stimulation and that LFOs decrease in amplitude with age. Schroeter et al. reported that the power spectral density peak in the VLF range in their study corresponded to the duration of the functional stimulation cycle [8]. In our study, task duration was 180 s. Hence, our results on VLFOs cannot be attributed to the frequency or duration of stimulus presentation.

The second aim of our study was to examine the effects of age and cognitive load on BP oscillations. Our results showed that increased cognitive load resulted in systemic changes; BP increased slightly in both young and older adults, and VLFOs of BP reduced in amplitude in young adults. We performed transfer function analysis to investigate the relationship between BP and [O_2Hb] oscillations in the VLF and LF range. In accordance with the literature, the coherence between BP and [O_2Hb] oscillations was found to be large [6, 30]. Phase shifts in the VLF range approached zero, but, consistent with other studies, the standard deviations were very large in both groups. The study of Pfurtscheller et al. [30] showed that phase shifts between cardiovascular oscillations and cerebral haemodynamic oscillations vary largely across individuals, but are relatively stable within one individual. In general, phase relationships have been more widely studied between BP and cerebral blood flow velocity (CBFV), with fewer studies also including [O_2Hb] (e.g. [20]). Due to effects of cerebral autoregulation, CBFV oscillations lead BP oscillations in the VLF range and, to a lesser extent, in the LF range, and they are in phase in the HF range. Because of the time delay between changes in CBFV and changes in [O_2Hb], this pattern differs for phase shifts between BP and [O_2Hb]; in the VLF range, where CBFV leads BP, oscillations in [O_2Hb] become more or less in

phase with BP due to the delay in [O_2Hb]. This may result in the large variation around zero we found in our study. In the LF range, our results showed that BP oscillations lead [O_2Hb] oscillations, which is in agreement with previous studies [6, 20, 28]. In accordance with the
192 results of Phillip et al. we did not find a clear effect of age on gain between BP and [O_2Hb] oscillations [31]. Taken together, we conclude that the relationship between BP and [O_2Hb] oscillations (cerebral autoregulation) was not affected by cognitive load and age.

We observed that both VLFOs of cerebral haemodynamics and BP declined by a similar magnitude with cognitive load in young adults. In view of the established relationship between BP and cerebral oscillations using transfer function analysis, this observation suggests – but does not prove – that this decrease in VLFOs is explained by the decrease in VLFOs of BP. In turn, this speculatively implies a systemic effect of cognitive load, possibly due to activation of the autonomic nervous system. However, in the older subjects, alterations in cerebral haemodynamic oscillations appeared less clearly dependent on BP oscillations. The origins of the slow haemodynamic oscillations are under debate and to date largely unclear (see, e.g., [32]). They may reflect either vasomotion or Mayer waves, which represent distinct but related phenomena. Vasomotion refers to tone oscillations in microvascular smooth muscle cells, resulting in spontaneous variation of the vascular diameter [33]. Mayer waves are defined as spontaneous oscillations in arterial BP, representing feedback oscillations of the baroreflex loop modulated by the sympathetic nervous system [34]. Previous studies have made a distinction between VLFOs and LFOs of cerebral haemodynamics, although no agreement exists on the frequency ranges. Therefore, it is unclear how power changes in the different frequency bands are linked to specific underlying physiological mechanisms. It has been proposed, however, that slow wave vasomotion (VLFOs) originates from the large arterioles (50 - 100 μm), whereas fast wave vasomotion (LFOs and HFOs) arises from terminal arterioles [35]. Neurogenic innervation occurs in the large arterioles, while the terminal arterioles lack such a neural supply [9]. Aging is accompanied by a degeneration of structure and function of the cerebral vasculature [10]. Schroeter et al. therefore hypothesized that a reduction of LFOs with aging suggests a decline in the activity of the microvascular smooth muscle cells together with increased vessel stiffness [8]. Our data are in line with this interpretation, since we found an aging-related reduction of VLFOs, LFOs and HFOs of cerebral haemodynamics. Furthermore, our results showed that VLFOs varied in power with cognitive load in young adults, but not in older adults. Since VLFOs may originate from large arterioles for which neurogenic innervation was shown, we speculate that the contribution of neurogenic activity to the regulation of regional cerebral blood flow is relatively lower in elderly, presumably due to vessel stiffness.

For the HF range we observed an aging-related decline of cerebral haemodynamic oscillations. Furthermore, it can be noticed that the spectral power peaks of BP in the HF range coincide with the frequency of stimulus presentation, which was approximately 0.28 Hz. No clear peaks in the HF range could be detected for [O_2Hb] oscillations, which is likely related to the inherently lower power of this signal. Respiratory cycles are a main contributor to HFOs of BP and cerebral haemodynamics. Additionally, the motor response may influence the HFOs via two mechanisms, although with a latency of several seconds [30, 36, 37]. First, neocortical structures involved in movement execution initiate heart rate changes via brainstem cardiovascular nuclei [38]. Second, the reafferent input from the kinaesthetic receptors evoked by the button press elicits a BP response [39]. Taken together, when the influence of respiration and motor execution is taken into account, it is plausible that participants were breathing in synchrony with stimulus presentation or button pressing. This may have influenced the results on HFOs of BP and cerebral haemodynamics.

Task-evoked systemic changes may confound the fNIRS signal [40]. A major challenge for signal analysis is to separate these systemic changes from the haemodynamic changes that are related to neural activity. Katura et al. quantified the contribution of systemic signals to LFOs (0.04 - 0.15 Hz) in cerebral haemodynamics under rest [4]. Using transfer entropy for analysis, they found that the contribution of systemic signals to LFOs of [O_2Hb] was 35 %; 20 % could be attributed to heart rate, 5 % to BP, while their common contribution was 10 %. For LFOs of [HHb] this was 7 %; 5 % could be attributed to heart rate, 1 % to BP, while their common contribution was 1 %. Katura et al. concluded that the origin of LFOs in cerebral haemodynamics may lie in the regulation of regional cerebral blood flow and energetic metabolism rather than due to the systemic regulation of the cardiovascular system. Task-evoked systemic fluctuations may however be substantial and may mask cerebral haemodynamic changes or lead to false positive findings [14, 41-43].

Several methods have been proposed to separate systemic contributions to the fNIRS signal from local cerebral haemodynamic contributions and these have been summarized in the paper of Kirilina et al. [42]. Methods include for example short-separation measurement to estimate extracranial contribution [44], statistical parametric mapping with the inclusion of systemic variables as regressors [45], or independent component analysis [46, 47]. Our current study not only stresses the importance of taking systemic variables into account in fNIRS signal analysis, but also indicates that the factors cognitive load and aging influence both systemic and cerebral haemodynamic contributions.

We recognize some limitations of our study. First, respiration was not recorded in this study. The observed HF spectral power peaks suggest that participants were breathing in synchrony with stimulus presentation or button pressing. For future research it is recommended to monitor respiration, or end tidal CO_2, to control for task induced changes in respiration, or hyperventilation. Second, the exact moment of a button press was not marked in the recorded signals. Therefore, we were not able to calculate the movement-triggered BP and heart rate fluctuations. However, the design of our study may not have allowed such a calculation due to the latency of the BP response. Third, we cannot quantify the exact contribution of systemic noise to the fNIRS signals. Transfer function analysis showed a relationship between BP and [O_2HB] oscillations, but the extent of the contribution remains unclear. Methods that are being used to separate systemic contributions to the fNIRS signal from local cerebral haemodynamic contributions have been mentioned above. For future research it is recommended to gain more insight in the sources of noise by for example co-registering skin blood flow.

The application of spectral analysis to study spontaneous oscillations in elderly may be useful for clinical practice. Previous studies demonstrated reduced spontaneous oscillations in patients with cerebral infarction [48], people at risk for atherosclerotic stroke [49] and people with cerebral microangiopathy [50], indicating vascular alterations such as vessel stiffness. Moreover, Schroeter et al. found that changes in the amplitude of the oscillations were tightly related to arterial hypertension and neuropsychological deficits [50]. Hence, spectral analysis, next to time course analysis, of the cerebral haemodynamic signal may be useful for early detection of vascular dementia or stroke.

To summarize, our fNIRS study showed that slow oscillations of cerebral haemodynamics and BP decline with aging. VLFOs are influenced by cognitive load in young adults, but not in older adults. These findings are presumably due to aging-related changes in the microvasculature such as vessel stiffness. Moreover, our results indicate that not only local vasoregulatory processes, but also systemic processes influence the cerebral haemodynamic signals. To conclude, the effects of age and BP should be taken into account in the analysis and interpretation of neuroimaging data that rely on blood oxygen levels.

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Chapter 9.

Cerebral perfusion in hypertensive elderly before and after antihypertensive treatment.

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ABSTRACT

Hypertension affects 20 % to 30 % of the world population and is the most prevalent modifiable risk factor for stroke. Several studies have demonstrated that antihypertensive drug therapy reduces the development of new coronary events, stroke and congestive heart failure in elderly persons. However, at short term antihypertensive drug therapy may also have disadvantages. Indications exist that hypertension impairs the mechanism of cerebral autoregulation itself. Furthermore, orthostatic hypotension is a common problem among elderly patients and could be attenuated by antihypertensive drug therapy. Hence, a higher risk for hypoperfusion occurs.

Herein, we report haemodynamic changes after antihypertensive treatment in three elderly patients with hypertension. In the three elderly patients, over the age of 70 years, with hypertension described in this case report it was shown that BP lowering did not result in a lowering of the CBFV. Also the cerebral autoregulatory functioning was preserved after 3 months of antihypertensive treatment. One patient did show increased orthostatic hypotension after antihypertensive treatment, but the ratio between BP and cerebral blood flow remained the same.

Overall, the findings of this case report suggest that it is save to apply antihypertensive treatment in elderly regarding brain perfusion.

INTRODUCTION

Hypertension affects 20 % to 30 % of the world population and is the most prevalent modifiable risk factor for stroke [1]. Long-standing hypertension may result in structural changes of the cerebral vessels, such as thickening of the vessel walls with narrowing of the lumen and hyalinosis of the media resulting in stiffness. The seventh report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (BP) considered that vascular risk begins with BP values of 115/75 mmHg, and that the risk doubles with each increase of 20/10 mmHg [2]. The relationship between BP and stroke risk has been demonstrated for all age groups studied. While the strength of the association becomes attenuated with increasing age, it is still strong and continuous among those aged 70 years or more. Several studies have demonstrated that antihypertensive drug therapy reduces the development of new coronary events, stroke and congestive heart failure in elderly persons [2]. Results from HYVET (Hypertension in the Very Elderly Trial) showed that, at 2-year follow-up, antihypertensive drug therapy reduced stroke by 30 %, all-cause mortality by 21 %, cardiovascular death by 23 % and heart failure by 64 % [3].

However, at short term antihypertensive drug therapy may also have disadvantages. Indications exist that hypertension impairs the mechanism of cerebral autoregulation itself, by shifting the ranges of the autoregulatory curve upwards [4]. This upwards shift may result in transient falls in cerebral blood flow when BP is lowered with antihypertensive drug treatment. Furthermore, orthostatic hypotension is a common problem among elderly patients [5] and could be attenuated by antihypertensive drug therapy. Hence, a higher risk for hypoperfusion occurs. The HYVET study did not present any indications for these disadvantages, however the population studied contains less comorbidities and is not representative for the Western population. Furthermore, systolic BP was only lowered to 150 mmHg [3].

Herein, we report haemodynamic changes after antihypertensive treatment in three elderly patients with hypertension.

METHOD

Three patients (1 man and 2 women), over the age of 70 years, with hypertension (systolic BP >150 mmHg) participated in research in our department. They had been carefully screened by a geriatrician including a medical history, physical examination and

electrocardiogram to exclude acute medical conditions or cardiovascular diseases other than hypertension. To ensure true hypertension, patients were asked to record their BP twice daily (approximately 8 AM and 8 PM) at home using a semiautomatic device.

At their first visit, none of the hypertensive patients had received antihypertensive therapy. They underwent the test protocol described below. After their visit, they received common antihypertensive treatment as endorsed by the Dutch CBO guidelines which has as goal to lower the systolic BP to below 140 mmHg. Patient came back for a follow-up measurement after 3 months of antihypertensive treatment.

The test protocol took place in the morning, at least 2 h after a light breakfast and 12 h after the last caffeinated beverage or alcohol, in a quiet, environmentally controlled laboratory with an ambient temperature of 22°C. Continuous BP (FinaPres Medical Systems, the Netherlands), three-lead ECG, cerebral blood flow velocity (CBFV) (Multi-Dop, Compumedics DWL, Germany), end-tidal CO2 (capnography BIOPAC Systems, Goleta, Ca.) and oxygenation index (HbDiff) (near-infrared spectroscopy, Oxymon Mk III, Artinis Medical System, The Netherlands) were recorded. After at least 10 min of rest in sitting position, a baseline measurement of 5 min was recorded during spontaneous respiration. Patients were asked to perform a single sit-to-stand protocol. They sat in a straight-backed chair and were asked to stand up. The protocol consisted of three trials of 2-min sitting followed by standing for 1 min. During all manoeuvres, patients were instructed to keep normal breathing and to avoid performing a Valsalva manoeuvre. Adherence to this was confirmed by inspecting the CO_2 waveforms and etCO_2 registrations. To investigate cerebral vasomotor reactivity (CVMR), a previously described protocol was used [6], consisting of a 30 s period of coached hyperventilation, followed by 2 minutes of spontaneous breathing. Next, subjects were asked to inhale a gas mixture containing 7 % CO_2, 21 % O_2 and 72 % N_2 through a tightly fitting mouthpiece until a stable plateau of CBFV had been reached. With this protocol a wide range of changes in etCO_2 can be obtained.

Data processing

All data were simultaneously recorded at 200 Hz. Post processing was performed using custom-written MATLAB scripts. Real time beat-to-beat mean values of BP and CBFV were calculated as waveform integration of the BP and CBFV signal within each cardiac cycle. To evaluate the beat-to-beat dynamics of BP, CBFV and HbDiff responses to acute posture changes in the single sit-stand protocol, we calculated the differences between the sitting

value (averaged over a period of 20 s) and the value at the nadir of BP (average of 5 values surrounding the nadir) for BP, CBFV and HbDiff.

The CVMR protocol evaluated changes in BP, CBFV, calculated cerebrovascular conductance index (CVCI) and HbDiff during the transitions from hypocapnia (induced by hyperventilation) to normocapnia and from normocapnia to hypercapnia (induced by 7 % CO_2 inhalation).

Because changes in CO_2 cause changes in BP, which in turn may directly affect CBFV, CVCI, expressed as CBFV changes divided by BP changes, was used to minimize the confounding effects of differences in BP response during CVMR testing between subjects on CVMR estimation [7].

Transfer function analysis was performed on the spontaneous oscillations in the baseline measurement as a measure for cerebral autoregulation using the method described by Zhang et al. [8] and reviewed by van Beek et al. [9]. The time series of mean BP and CBFV were first subdivided into 950-point segments with 50 % overlap for spectral estimation. This process resulted in five segments of data for the segment periodogram average. Fast Fourier transforms were implemented with each Hanning-windowed segment and averaged to quantify the transfer function. For quantification of CA the positive phase shift and the gain between BP and CBFV were quantified as means of the following frequency bands: very low frequency: 0.02 - 0.07 Hz; low frequency: 0.07 - 0.15 Hz; and high frequency: 0.15 - 0.35 Hz. The transfer function analysis is based on the high-pass filter model of cerebral autoregulation, wherein BP oscillations at lower frequencies are buffered better than higher frequencies, leading to lower gain (better damping) in these lower frequencies. Also, a characteristic of this model is that the counteractive actions of autoregulation lead to a phase shift between CBF and BP in these lower frequencies [9].

RESULTS

Demographic baseline data and follow up data for the three patients are summarized in Table 1. In all three patients, antihypertensive treatment lowered BP (average of -19 mmHg). Mean CBFV rose after antihypertensive treatment (average of 12 cm/s).

The haemodynamic changes for the single sit-stand manoeuvres are shown in Figure 1. The posture change from sit to stand evoked transient reductions in BP. Patient 1 and 2 did not show any differences in BP. Only patient 3, whom showed a greater fall in BP after

Table 1. Demographic data before and after treatment.

SBP = systolic blood pressure. DBP = diastolic blood pressure. HR = heart rate. MCBFV = mean cerebral blood flow velocity.

	patient 1		**patient 2**		**patient 3**	
Age (years)	70		78		71	
Sex (male/female)	male		female		female	
Average home measurements	**before treatment**	**after treatment**	**before treatment**	**after treatment**	**before treatment**	**after treatment**
SBP morning (mmHg)	151	130	166	147	163	147
DBP morning (mmHg)	80	69	90	81	72	69
HR morning (bpm)	77	87	65	61	50	51
SBP evening (mmHg)	169	141	165	152	150	144
DBP evening (mmHg)	88	72	91	82	74	65
HR evening (bpm)	85	84	70	68	56	54
MCBFV (cm/s)	41	48	35	57	35	41

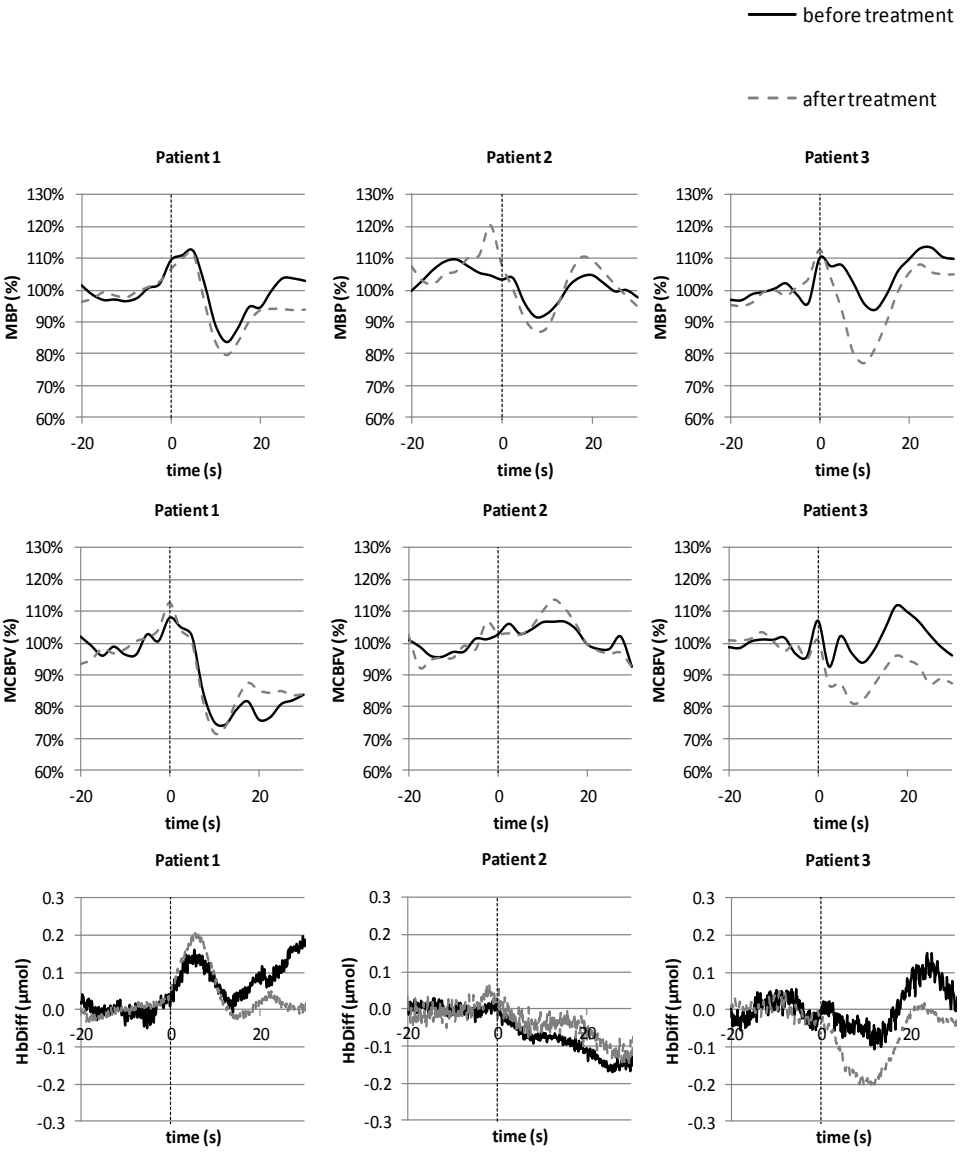

Figure 1. Haemodynamics during sit-to-stand manoeuvre before and after treatment. Results are shown for 20 seconds before standing up till 30 seconds after standing up. MBP = mean blood pressure. MCBFV = mean cerebral blood flow velocity. HbDiff = oxygenation index.

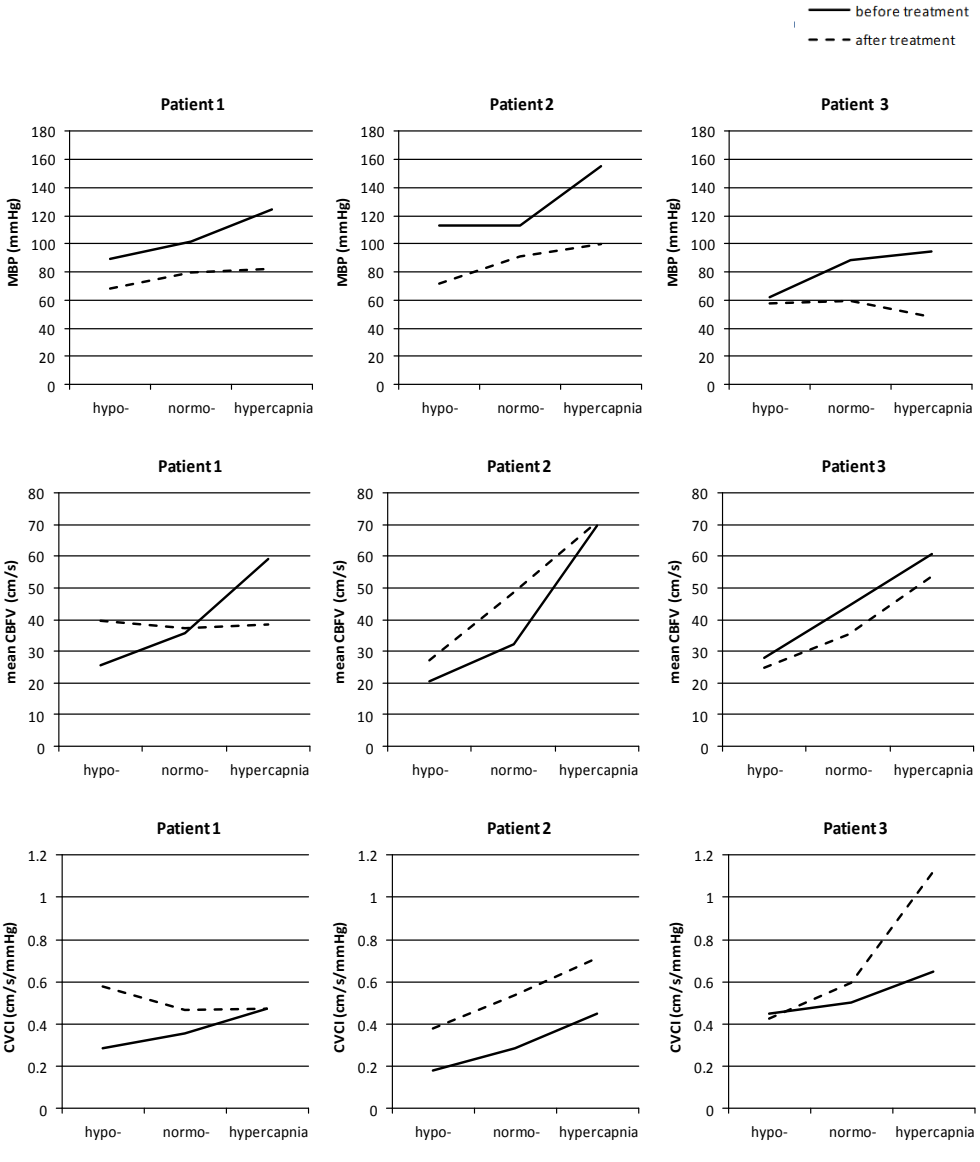

Figure 2. Haemodynamics during cerebrovasomotor reactivity test.

The cerebral vasomotor reactivity test consisted of a 30 s period of coached hyperventilation (hypocapnia), followed by 2 minutes of spontaneous breathing (normocapnia). Next, subjects were asked to inhale a gas mixture containing 7% CO2, 21% O2 and 72% N2 through a tightly fitting mouthpiece to create hypercapnia (hypercapnia). MBP = mean blood pressure. MCBFV = mean cerebral blood flow velocity. CVCI = cerebrovascular conductance index.

antihypertensive treatment, had a greater decrease in mean CBFV and HbDiff after antihypertensive treatment. The haemodynamic changes for CVMR are shown in Figure 2. Before antihypertensive treatment, all patients showed a lowered CBFV during hypocapnia and an increased CBFV during hypercapnia. After antihypertensive treatment, this cerebrovascular reactivity phenomenon was preserved in patient 2 and 3. Patient 1 showed no changes in CBFV during hypo- and hypercapnia after antihypertensive treatment.

Transfer function analysis revealed that the high-pass filter characteristics of cerebral autoregulation were remained after antihypertensive treatment.

DISCUSSION

Although tight BP control is known to have beneficial impacts in patients with hypertension [10, 11], physicians are cautious with prescribing anti-hypertensive treatment in elderly due to the unknown effect on cerebral perfusion. In the three elderly patients with hypertension described in this case report it was shown that BP lowering did not result in a lowering of the CBFV. In contrast, even a rise in CBFV was observed.

Hypertension affects nearly a third of the general population, which is about one billion people worldwide [12], and is an important risk factor for cardiovascular disease. The prevalence of hypertension increases dramatically with age, such that as much as 77 % of subjects over age 70 are hypertensive [13]. If hypertension is adequately treated it is possible to minimize the risk of heart attack, heart failure, stroke and kidney failure. However, in severe hypertension the cerebral vessels are narrowed with thickened walls and hyalinosis of the media results in stiffness, which may result in a need for higher perfusion pressures for the brain and a lower ability for cerebral autoregulatory vasodilatation [4]. Lowering the BP with antihypertensive treatment may thus make hypertensive patients more vulnerable to cerebral hypoperfusion. The concern that anti-hypertensive treatment may lead to hypoperfusion of the brain is reflected in the low number of elderly patients with hypertension who receive treatment. Only a third of hypertensive patients aged above 70 years receives treatment and has adequate controlled BP (defined as a systolic BP ≤ 140 mmHg); another third receives treatment but has inadequate control of hypertension, the final third is untreated [13]. This lack of knowledge may unnecessarily expose patients in need for antihypertensive treatment to a higher risk for i.e. stroke.

Several studies have investigated the negative effects of BP lowering on morbidity, quality of

life and mortality in patients of middle age with moderate hypertension [14, 15]. Thus far, antihypertensive treatment does not seem to have a negative influence on brain blood flow. However, with advancing age, evidence is scarce and less clear and concern for treatment-related harm increases.

Although aging is associated with changes in cerebrovascular haemodynamics [16-18], recent research has shown that the cerebral autoregulatory functioning is unaffected in normotensive aging [19]. In this case report, we have shown that in these hypertensive elderly, aged between 70 and 80 years, also the cerebral autoregulatory functioning was preserved. As the primary goal of cerebral autoregulation is to maintain an adequate cerebral perfusion despite changes in BP, an adequate cerebral autoregulatory functioning predicts that cerebral blood flow would be preserved following BP lowering with antihypertensive treatment. After 3 months of antihypertensive treatment, the BP lowering did, indeed, not result in a lowering of the CBFV. In contrast, even a rise in CBFV was observed. One patient did show increased orthostatic hypotension after antihypertensive treatment, but the ratio between BP and cerebral blood flow remained the same. In another patient, after antihypertensive treatment, no decrease nor increase in cerebral blood flow was seen during hypo- and hypercapnia, respectively. This suggests a blunted cerebral vasomotor reactivity.

We need to mention that the use of CBFV with transcranial Doppler to estimate actual blood flow requires that the vessel diameter remains unchanged and that the perfusion territory of a given artery remains constant [20]. Numerous studies have shown that middle cerebral artery diameter in humans remains relatively constant under a variety of haemodynamic conditions [21-23]. However, it is possible that the medications used to induce hypertension may have had a direct effect on the cerebral vessels confounding our results. During hypotension, hypocapnia and hypercapnia in healthy subjects and neurosurgical patients, diameter of the middle cerebral artery was unchanged or showed less than 4 % change [21, 23]. Studies in hypertensive patients are lacking. Measurements of isolated middle cerebral artery from spontaneously hypertensive rats indicate a 10 % change in diameter with large changes in mean BP from 40 to 140 mmHg, suggesting that in our study, small changes in MCA diameter cannot be excluded. However, such changes would have led to a finding of reduced CBFV after treatment. The relatively narrower the lumen of the middle cerebral artery in hypertensive patients before treatment might increase CBFV, overestimating CBF. Subsequently, we doubt that, in this case report, the found rise in CBFV after treatment is caused by a significant effect of the medication on the cerebral vessels.

Overall, the findings of this case report have clinical significance since they suggest that it is save to apply antihypertensive treatment in elderly patients with regard to the level of cerebral blood flow and cerebral autoregulatory functioning. Antihypertensive treatment would have the benefit of reducing the development of (new) coronary events, stroke and congestive heart failure [2]. However, as these results are only obtained in a small sample of three patients, a larger observational trial is needed to confirm our findings. Furthermore, as this case report is inconclusive on a possible causal relationship between blunted cerebral vasomotor reactivity and anti-hypertensive treatment further studies on this topic are warranted.

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Chapter 10.

Geriatric hypotensive syndromes are not explained by cardiovascular autonomic dysfunction alone

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ABSTRACT

Background

Though highly prevalent, the pathophysiology of orthostatic hypotension (OH), postprandial hypotension (PPH) and carotid sinus hypersensitivity (CSH) are rarely studied together. Therefore, we conducted such a comprehensive study focusing on the common role of the cardiovascular autonomic system. We hypothesized that in geriatric patients, OH, PPH and CSH are manifestations of cardiovascular autonomic dysfunction and investigated state-of-the-art cardiovascular autonomic function indices in a group of geriatric falls or syncope patients.

Methods

In a cross-sectional study of 203 consecutive eligible falls clinic patients, we compared heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity (BRS) as potential autonomic function determinants of the three different hypotensive syndromes.

Results

OH, PPH and CSH were diagnosed in 53%, 57% and 50% of the patients, respectively. In a population relevant for geriatric practice, we found no differences in HRV, BPV and BRS between patients with and without OH, with and without PPH, and with and without CSH, respectively, nor between patients with and without falls, dizziness, or syncope as presenting symptom, respectively.

Conclusions

In geriatric patients with hypotensive syndromes, cardiovascular autonomic function as measured by HRV, BPV and BRS is comparable to patients without such syndromes. These findings argue against a single or dominant etiological factor, that is, cardiac autonomic dysfunction and underline the structured, broad and multifactorial approach to elderly patients with falls and/or syncope as proposed in the current evidence-based syncope guidelines.

INTRODUCTION

Heart rate variability (HRV), blood pressure variability (BPV) and baroreceptor sensitivity (BRS) are established measures of cardiovascular autonomic function in research [1-3]. The clinical relevance of these measures has also been shown, as diminished BRS, diminished HRV and increased BPV have prognostic significance for mortality and cardiovascular risk [4-6].

Orthostatic hypotension (OH), postprandial hypotension (PPH) and carotid sinus hypersensitivity (CSH) are disorders of blood pressure (BP) regulation with high prevalence in elderly patients [7-12]. OH is predominantly seen as a disorder of autonomic failure, and PPH and CSH are classified as reflex or neurally mediated syncope [13]. The cardiovascular autonomic system plays an important role in the distribution of blood volume and the regulation of BP [8, 14-17], and failure of this system might play an important role in the aetiology and pathophysiology of these hypotensive syndromes.

Therefore, we hypothesized that commonly available and non-invasive autonomic function indices of HRV, BPV and BRS are different in patients with and without OH, PPH and CSH.

METHODS

Study Population

This study included 242 consecutive patients, who visited the geriatric outpatient falls and syncope clinic of the Radboud University Nijmegen Medical Centre because of falls, dizziness and/or syncope. This test protocol was part of the standard diagnostic work-up. Patients were excluded if they were unable to follow the instructions due to delirium, psychosis or very severe dementia (Clinical Dementia Rating 3), if they were unable to stand for 10 min or drink a test meal of 200 mL within 10 min. Furthermore, patients were excluded for CSH testing in case of a recent (less than 3 months) myocardial infarction, transient ischemic attack, or stroke, respectively, a medical history of ventricular tachyarrhythmias or the presence of a carotid bruit [18].

The protocol conformed to the Declaration of Helsinki and informed consent was asked verbally prior to the tests.

Baseline Assessment

Preceding OH, PPH and CSH testing, patients underwent a complete medical history and
218 physical examination. Baseline characteristics (age, gender, body mass index [BMI], systolic BP [SBP], diastolic BP [DBP] and heart rate [HR]) were measured. The presence and severity of comorbidity was recorded using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G), ranging from 0 to 56 [19]. For medical history, we used the following disease groups: dementia; depression or anxiety disorder; chronic obstructive pulmonary disease; diabetes mellitus; Parkinson's disease or disorders with parkinsonism; cardiovascular disease (CVD) including myocardial infarction, angina pectoris, heart failure, peripheral vascular disease, aneurysm of the aorta, stroke, and transient ischemic attack; hypertension; and malignancy.

Test Protocol

Patients fasted overnight and medication was withheld from midnight the night before. All tests were performed in the morning or afternoon. During the tests, BP and HR were constantly measured using a beat-to-beat finometer (FinaPres [20]) and a three-lead electrocardiogram (ECG). BP was measured at the nondominant arm, which was held at heart level with a sling.

The test protocol started with the OH test, followed by the PPH test and finished with the carotid sinus massage (CSM). Before testing and at each time point during tests (OH test every minute and PPH test every 5 min), BP and HR were determined. The reported BP values are averages of 20 heartbeats (10 before and 10 after each time point). For CSH testing, BP values were determined by taking the average of 3 heartbeats around the lowest BP value during or in a 30-s period after CSM. From the ECG registration, the R–R interval before and the longest interval during or in a 30-s period after CSM were documented. At each time point, symptoms of possible cerebral hypoperfusion, like dizziness, light-headedness, fatigue, or loss of consciousness were noted.

Orthostatic hypotension test

After a 10-min resting period in the supine position, patients were asked to stand up and remain standing for 10 min. Baseline values of BP and HR were defined as the average during the 60 s before rising. OH was defined as a SBP decline of 20 mmHg or more or a DBP decline of 10 mmHg or more within 3 min of standing from supine [13].

Postprandial hypotension test

The patient was asked to drink a test meal within 10 min in sitting position. The test meal is a solution of 100 mL glucose syrup and 100 mL lactose-free milk, containing 292 kcal, 65 g carbohydrates, 2 g fat and 4 g protein. The meal temperature was 20 - 25°C [21]. After drinking the meal, BP and HR were monitored during 75 min. PPH was defined as a SBP decline of 20 mmHg or more within 75 min after a meal [7, 9].

Carotid sinus massage

CSM was performed in supine position after 5 min rest. Firm, longitudinal massage was performed for 5 s over the site of maximal pulsation of the right carotid artery and repeated on the left side when SBP and HR were normalized. If no significant response in SBP or HR was obtained with supine CSM, the procedure was repeated with the patient tilted at 70° on a tilt table with footplate. CSH is defined as an interruption of heart beat more than at least 3 s or as a decrease of SBP of 50 mmHg or more.

Assessment of HRV, BPV and BRS

Data were analysed with custom-written software in Matlab (version R2010b, the MathWorks Inc., Natick, Massachusetts). The 10-min resting period in supine position was used to select an appropriate period to determine the measures of HRV, BPV and BRS. Not necessarily, the same periods were used to calculate HRV, BPV, or BRS.

Heart rate variability

The standard deviation of all normal beat intervals (SDNN-HRV) and the square root of the mean-squared difference of successive beat intervals (RMSSD-HRV) were used for conventional time domain measurements [1]. The data were linearly detrended and filtered with a third-order Butterworth filter with a cut-off frequency of 0.03 Hz and resampled to 4 Hz using linear interpolation. Artefact-free data series of 100 s were used. With spectral analysis, the power of HRV was calculated in the low (LF-HRV; 0.04 - 0.15 Hz) and high (HF-HRV; 0.15 - 0.4 Hz) frequency band as conventional frequency domain measurements [1]. Also, the ratio of LF-HRV and HF-HRV was determined (LF/HF-HRV).

Blood pressure variability

The BPV was also analysed in the time and frequency domain. Only the systolic BP was used. The standard deviation of BP (SD-SBP) and the square root of the mean-squared difference of

successive BP beats (RMSSD-SBP) were used for conventional time domain measurements. The data were linearly detrended and filtered with a third-order Butterworth filter with a cut-off frequency of 0.05 Hz and resampled to 1 Hz using linear interpolation. Only
220 uninterrupted data series of at least 50 s were used for the spectral analysis. With spectral analysis, the power of BPV was calculated in the low (LF-SBP; 0.05 - 0.15 Hz) and high (HF-SBP; 0.15 - 0.4 Hz) frequency band. These frequency bands were defined largely in line with the standards of the European Society of Cardiology for HRV, meaning LF = 0.04 - 0.15 Hz and HF = 0.15 - 0.4 Hz [1]. However, because a filter with cut-off frequency 0.05 Hz was used as recommended by Bernardi et al. [22], LF for BPV was chosen between 0.05 and 0.15 Hz.

Baroreflex sensitivity

BRS was quantified in the time domain with the sequence and the standard deviation method (SD-BRS) and in the frequency domain with spectral analysis. The sequence method was applied for positive (SQ-BRS+) and negative (SQ-BRS−) sequences [23]. The SD-BRS method calculates the BRS by dividing the standard deviation of the heartbeat interval by the standard deviation of SBP (SD-BRS). The SD-BRS is an easy, robust and reliable method to calculate BRS [22].

The data were linearly detrended and filtered with a third-order Butterworth filter with a cut-off frequency of 0.05 Hz and resampled to 1 Hz using linear interpolation. Data series of at least 50 s were used. In the frequency domain, BRS is calculated in the low (0.05 - 0.15 Hz) and high (0.15 - 0.4 Hz) frequency band by dividing the spectral power of the heartbeat interval through the spectral power of SBP, only if the coherence is more than 0.5 [24]. Heartbeat intervals were derived from the BP peaks to overcome artefacts in the ECG [25].

STATISTICAL ANALYSIS

We compared the patients with and without OH, PPH and CSH, respectively. We used only those patients who completed all three tests. Because there is frequent coexistence of multiple hypotensive syndromes in the same patient, we also compared patients with no single hypotensive syndrome versus one, two or three hypotensive syndromes. We also compared patients with and without falls, dizziness or syncope as presenting symptom. Finally, we made a comparison between patients who presented with falls and patients who presented with syncope.

Baseline characteristics of the patients were compared with independent sample Student's *t* tests or one-way analysis of variance and chi-square (χ^2) statistics. Results are presented as mean ± SD or percentages.

Normality of HRV, BPV and BRS measures was tested with the Kolmogorov–Smirnov test. If the data were not normal distributed they were logarithmically transformed and tested again for normality. None of the HRV, BPV and BRS measures had a normal distribution. After logarithmic transformation, all data were normal distributed except for the SQ-BRS+, SQ-BRS– and SD-SBP data. Means were compared with independent sample Student's *t* tests or one-way analysis of variance for normal distributed data; otherwise, Mann-Whitney's *U* tests or Kruskal-Wallis tests were used. Because we compared 14 different measures of cardiovascular autonomic function, we choose an alpha of 0.01, instead of 0.05 to correct for multiple testing. Results for HRV, BPV and BRS measures are presented as medians with the 25th - 75th percentile range. All analyses were performed using the SPSS software version 16 for windows (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline Characteristics

Of the 242 patients, 203 (84 %) patients completed all three tests. CSM was not performed in 34 patients because they met the exclusion criteria. Of five other patients, there was no value for OH and/or PPH tests due to unreliable measurements (no good signal). Of these 203 patients, 53 %, 57 % and 50 % were diagnosed with OH, PPH and CSH, and 87 % patients were diagnosed with at least one or more hypotensive syndromes.

Baseline characteristics of patients with and without different hypotensive syndromes were compared (Table 1). Compared with the patients without OH, the patients diagnosed with OH had lower BMIs and had higher mean baseline SBP ($p < .05$). The patients with PPH were older, had higher baseline SBPs, and had more frequently a medical history of CVD ($p < .05$). The patients with CSH had lower BMIs, presented less often with dizziness and used less angiotensin-converting enzyme inhibitors or angiotensin-II receptor antagonists compared with those without CSH ($p < 0.05$). There were no differences in comorbidity (CIRS-G) nor the number of drugs used.

Table 1. Comparison of the baseline demographic and clinical characteristics of the patients with and without orthostatic hypotension, postprandial hypotension, and carotid sinus hypersensitivity.

The results are reported as means ± standard deviations or numbers (percentages). OH, orthostatic hypotension; PPH, postprandial hypotension; CSH, carotid sinus hypersensitivity; age(years); BMI, body mass index(kg/m^2); SBP, systolic blood pressure(mmHg); DBP, diastolic blood pressure(mmHg); HR, heart rate(beats per minute); CIRS-G, Cumulative Illness Rating Scale for Geriatrics; COPD, chronic obstructive pulmonary disease; CVD, cardio-vascular disease; ACE/AT2, angiotensin-converting enzyme inhibitors or angiotensin-II receptor antagonists; CCB, calcium channel blockers. ***** significant differences ($p<0.05$) between patients with and without OH; **†** significant differences ($p<0.05$) between patients with and without PPH; **‡** significant differences ($p<0.05$) between patients with and without CSH.

Variable	**OH– (n=95)**	**OH+ (n=108)**	**PPH– (n=87)**	**PPH+ (n=116)**	**CSH– (n=102)**	**CSH+ (n=101)**
Age	76.9±8.6	78.4±7.4	75.6±8.0	79.3±7.6**†**	76.7±9.0	78.7±6.7
BMI	27.3±4.9	26.0±4.2*****	27.3±4.7	26.1±4.4	27.2±4.8	26.0±4.2**‡**
Female, n (%)	55(58%)	74(69%)	59(68%)	70(60%)	63(62%)	66(65%)
Baseline SBP	162±24	171±25*****	160±22	172±26**†**	164±25	169±25
Baseline DBP	78±11	80±13	79±12	79±12	79±12	79±12
Baseline HR	67±11	68±12	68±12	67±11	68±12	68±11
CIRS-G total score	10.6±4.6	11.3±4.4	10.5±4.3	11.3±4.6	10.9±4.4	10.9±4.5
Presenting symptom						
Falls	79(83%)	96(90%)	74(86%)	101(87%)	90(89%)	85(84%)
Dizziness	48(51%)	55(51%)	41(48%)	62(53%)	61(60%)	42(42%)**‡**
Syncope	29(31%)	37(35%)	28(33%)	38(33%)	36(36%)	30(30%)
Symptomatic	-	67(62%)	-	46(40%)	-	51(51%)

Table 1. Continued

Variable	OH– (n=95)	OH+ (n=108)	PPH– (n=87)	PPH+ (n=116)	CSH– (n=102)	CSH+ (n=101)
Medical history						
Dementia	6(6%)	6(6%)	4(5%)	8(7%)	4(4%)	8(8%)
Depression or anxiety	15(16%)	21(19%)	20(23%)	16(14%)	19(19%)	17(17%)
COPD	16(17%)	19(18%)	15(17%)	20(17%)	16(16%)	19(19%)
Diabetes mellitus	13(14%)	16(15%)	9(10%)	20(17%)	18(18%)	11(11%)
Parkinson(ism)	4(4%)	6(6%)	3(3%)	7(6%)	7(7%)	3(3%)
CVD	31(33%)	42(39%)	20(23%)	53(46%)†	37(36%)	36(36%)
Hypertension	42(44%)	50(46%)	37(43%)	56(48%)	53(52%)	40(40%)
Malignancy	15(16%)	25(23%)	14(16%)	26(22%)	19(19%)	21(21%)
Medication use						
Polypharmacy	64(67%)	76(70%)	59(68%)	81(70%)	67(66%)	73(72%)
Beta blockers	35(37%)	44(41%)	34(39%)	45(39%)	46(45%)	33(33%)
ACE/AT2	26(27%)	43(40%)	26(30%)	43(37%)	43(42%)	26(26%)‡
CCB	11(12%)	19(18%)	13(15%)	17(15%)	16(16%)	14(14%)
Diuretics	31(33%)	39(36%)	30(35%)	40(35%)	39(38%)	31(31%)
Nitrates	10(11%)	15(14%)	8(9%)	17(15%)	15(15%)	10(10%)

Table 2. Comparison of heart rate variability, blood pressure variability and baroreflex sensitivity measures between patients with and without orthostatic hypotension, postprandial hypotension and carotid sinus hypersensitivity.

Results are reported as medians(25th-75th percentiles).

Heart rate variability: SDNN = standard deviation heart rate(ms); RMSSD = root mean square successive difference of heartbeats(ms); LF = low frequency power HRV($1x10^3$ms2); HF = high frequency power HRV ($1x10^3$ms2); LF/HF = ratio of LF-HRV and HF-HRV(-);

Blood pressure variability: SD = standard deviation systolic blood pressure (SBP) (mmHg); RMSSD = root mean square successive difference SBP(mmHg); LF = low frequency SBP(mmHg); HF-SBP = high frequency SBP (mmHg);

Baroreflex sensitivity (BRS): SQ+ = sequence method BRS for positive sequences(ms/mmHg); SQ- = sequence method BRS for negative sequences(ms/mmHg); SD = standard deviation BRS(ms/mmHg); LF = low frequency BRS(ms/mmHg); HF = high frequency BRS(ms/mmHg).*:p<0.01

	Heart rate variablity				
	SDNN	RMSSD	LF	HF	LF/HF
OH–	60(37-86)	51(26-86)	0.7(0.2-1.6)	0.7(0.1-1.9)	0.9(0.7-2.0)
n	86	86	84	84	84
OH+	49(30-88)	37(21-84)	0.4(0.1-2.3)	0.3(0.1-1.6)	1.2(0.6-1.8)
n	93	93	88	88	88
PPH–	50(27-72)	48(23-79)	0.8(0.1-1.4)	0.4(0.1-2.0)	1.0(0.7-2.5)
n	74	74	71	71	71
PPH+	53(34-89)	43(24-83)	0.4(0.2-1.7)	0.5(0.1-1.7)	1.0(0.7-2.0)
n	105	105	101	101	101
CSH–	56(31-90)	51(23-89)	0.8(0.1-1.8)	0.6(0.1-2.6)	1.0(0.7-2.3)
n	92	92	89	89	89
CSH+	49(33-76)	40(23-73)	0.4(0.2-1.2)	0.4(0.1-1.5)	1.0(0.7-1.9)
n	87	87	83	83	83

Table 2. Continued

	Blood pressure variability			
	SD	RMSSD	LF	HF
OH–	11.1(8.3-13.8)	6.2(4.3-8.7)	3.0(1.5-5.2)	3.2(1.7-8.3)
n	95	95	68	68
OH+	10.7(8.9-15.4)	7.2(5.3-11.3)	3.4(1.9-7.9)	4.9(2.5-10.2)
n	108	108	81	81
PPH–	11.5(9.0-15.2)	6.6(4.7-9.3)	2.5(1.5-5.4)	3.0(1.8-7.0)
n	87	87	67	67
PPH+	10.6(8.4-14.3)	6.7(4.8-9.9)	3.5(1.9-7.6)	4.9(2.5-10.8)
n	116	116	82	82
CSH–	11.0(8.0-14.5)	6.5(4.5-9.0)	3.5(1.7-7.9)	4.5(2.1-9.8)
n	102	102	81	81
CSH+	10.7(9.2-15.3)	7.2(4.9-10.3)	2.9(1.4-5.2)	3.9(1.9-7.7)
n	101	101	68	68

	Baroreflex sensitivity				
	SQ+	SQ-	SD	LF	HF
OH–	5.5(2.4-8.2)	9.3(4.0-13.3)	7.7(5.1-14.3)	7.0(3.6-12.7)	7.6(4.7-15.0)
n	95	95	95	50	61
OH+	3.5(1.5-5.5)	7.1(3.3-11.7)	7.4(3.8-13.7)	5.7(3.5-9.0)	5.7(3.4-10.6)
n	108	108	108	39	56
PPH–	3.6(1.3-7.2)	7.2(3.3-12.0)	7.9(4.3-14.3)	6.5(3.3-13.3)	7.0(3.2-17.4)
n	87	87	87	38	44
PPH+	4.6(2.2-7.2)	8.4(4.2-12.1)	7.3(4.1-13.7)	6.2(3.6-9.1)	6.3(3.8-11.3)
n	116	116	116	51	73
CSH–	4.9(2.2-8.3)	7.8(3.7-13.0)	8.3(5.2-14.0)	6.6(3.3-13.7)	6.6(3.6-15.5)
n	102	102	102	42	55
CSH+	3.8(1.8-5.9)	7.8(3.8-11.8)	6.9(3.4-14.2)	6.1(3.7-10.2)	6.8(3.8-11.0)
n	101	101	101	47	62

Table 3. Comparison of heart rate variability, blood pressure variability and baroreflex sensitivity measures between patients with and without falls, dizziness and syncope

Results are reported as medians(25th-75th percentiles).

Heart rate variability: SDNN = standard deviation heart rate(ms); RMSSD = root mean square successive difference of heartbeats(ms); LF = low frequency power HRV($1x10^3$ms2); HF = high frequency power HRV ($1x10^3$ms2); LF/HF = ratio of LF-HRV and HF-HRV(-);

Blood pressure variability: SD = standard deviation systolic blood pressure (SBP) (mmHg); RMSSD = root mean square successive difference SBP(mmHg); LF = low frequency SBP(mmHg); HF-SBP = high frequency SBP (mmHg);

Baroreflex sensitivity (BRS): SQ+ = sequence method BRS for positive sequences(ms/mmHg); SQ- = sequence method BRS for negative sequences(ms/mmHg); SD = standard deviation BRS(ms/mmHg); LF = low frequency BRS(ms/mmHg); HF = high frequency BRS(ms/mmHg).*:p<0.01

	Heart rate variablity				
	SDNN	RMSSD	LF	HF	LF/HF
OH–	50(33-111)	44(27-89)	0.4(0.2-1.9)	0.4(0.2-2.0)	1.2(0.6-2.5)
n	23	23	22	22	22
OH+	53(32-86)	43(23-82)	0.6(0.1-1.5)	0.4(0.1-1.9)	1.0(0.7-2.1)
n	155	155	149	149	149
PPH–	58(37-91)	55(28-84)	0.7(0.2-1.6)	0.7(0.1-1.9)	1.0(0.6-2.5)
n	87	87	84	84	84
PPH+	48(30-77)	39(21-71)	0.4(0.2-1.6)	0.3(0.1-1.8)	0.9(0.7-1.9)
n	91	91	87	87	87
CSH–	51(34-84)	45(24-82)	0.6(0.2-1.4)	0.4(0.1-1.6)	1.0(0.7-2.1)
n	121	121	115	115	115
CSH+	59(26-88)	37(20-86)	0.4(0.1-1.6)	0.5(0.1-2.7)	1.0(0.7-2.3)
n	57	57	56	56	56

Table 3. Continued

	Blood pressure variability			
	SD	RMSSD	LF	HF
OH–	11.2(7.8-16.3)	6.4(3.9-8.8)	2.8(1.6-5.3)	3.2(1.7-5.9)
n	27	27	18	18
OH+	10.8(9.0-15.1)	6.7(4.9-9.9)	3.3(1.7-6.7)	4.2(2.1-9.3)
n	175	175	130	130
PPH–	10.8(9.2-14.1)	6.6(4.9-9.2)	2.9(1.4-7.6)	4.0(1.9-8.6)
n	99	99	71	71
PPH+	11.0(8.3-15.6)	6.4(4.5-10.6)	3.4(1.9-5.9)	4.1(2.1-9.9)
n	103	103	77	77
CSH–	11.2(9.4-14.9)	6.7(4.9-9.3)	3.0(1.6-5.9)	3.9(1.9-8.6)
n	136	136	102	102
CSH+	9.8(7.4-15.8)	6.6(4.5-11.6)	4.4(1.7-9.6)	5.3(2.2-10.8)
n	66	66	46	46

	Baroreflex sensitivity				
	SQ+	SQ-	SD	LF	HF
OH–	5.2(2.4-7.8)	9.0(5.4-11.5)	7.6(4.2-11.7)	5.4(3.7-14.3)	7.4(5.2-17.9)
n	27	27	27	14	19
OH+	4.3(1.8-7.1)	7.6(3.7-12.1)	7.6(4.2-14.3)	6.6(3.5-10.2)	6.4(3.3-12.1)
n	175	175	175	75	98
PPH–	3.9(1.8-7.1)	7.4(3.9-11.2)	7.6(4.1-14.3)	7.0(3.7-12.5)	6.6(3.3-13.4)
n	99	99	99	43	57
PPH+	4.5(2.1-7.4)	8.6(3.7-13.3)	7.6(4.9-14.1)	5.4(3.2-10.3)	6.8(4.0-10.9)
n	103	103	103	46	60
CSH–	4.4(2.1-7.1)	7.7(3.9-12.2)	7.2(4.1-12.8)	7.0.6(3.7-	6.8(3.7-12.2)
n	136	136	136	61	81
CSH+	4.3(1.8-8.0)	8.1(3.3-11.6)	8.1(5.5-14.4)	5.9(3.3-9.1)	6.1(3.7-15.9)
n	66	66	66	28	36

Measures of HRV, BPV and BRS

No differences were found in the different measures of HRV, BPV and BRS between patients
228 with or without OH, PPH and CSH, respectively (Table 2). Similar results were found when patients with no versus one, two or three hypotensive syndromes were compared (data not shown). Also, no differences were found in the different measures of HRV, BPV and BRS between patients with or without falls, dizziness and syncope, respectively (Table 3). There were also no differences between patients who presented with falls and patients who presented with syncope (data not shown).

Finally, 20 patients had delayed OH, defined as onset of OH after the third minute. These patients were categorized among the "no OH" patients (n = 94) in our study. Compared with the n = 74 patients without OH and without delayed OH, they did not differ in HRV, BPV and BRS (data not shown).

DISCUSSION

The main finding of this study is that there were no differences in HRV, BPV and BRS between geriatric patients with and without hypotensive syndromes. We used extensive non-invasive, easy available and state-of-the-art indices of HRV, BPV and BRS [1, 22-24]. Most of these indices reflect primarily vagal activity, but LF-HRV and LF/ HF-HRV are also thought to reflect sympathetic activity or to reflect the sympathovagal balance [1].

Previous studies which examined the relationship between hypotensive syndromes and measures of HRV, BPV and BRS have reported conflicting results. In older persons, there was an association between diminished BRS with BP fall during OH testing [26, 27]. However, no difference in spectral measures of HRV and BPV was found between patients with normal and poor orthostatic tolerance [28]. During intraduodenal glucose administration for PPH testing, an association between diminished BRS with SBP fall was found [29]. Spectral measures of HRV were lower [30] and systolic BPV [31] was higher in patients with versus without PPH, but the latter study could not replicate the differences in HRV measures [31]. BRS was found to be higher [8], the same [32], or lower [33] between patients with and without CSH. Spectral measures of HRV were higher in patients with CSH compared with patients without CSH [8]. In another study, no differences were found in systolic BPV measures between patients with and without CSH [34]. An explanation for these conflicting data is the difference in used methods and in diagnoses, severity of illnesses and functional capacity between study

populations. Another explanation is the diversity of methods used to measure HRV, BPV and BRS in either the time or frequency domain [35]. Age-related normal values for different measures of HRV, BPV and BRS are lacking, which makes comparison between populations difficult [1].

Our data add to the literature because for the first time all three hypotensive syndromes are meticulously characterized together in one population of consecutive older patients who visited a geriatric outpatient falls and syncope clinic. Cardiovascular autonomic dysfunction, as measured with multiple, non-invasive measures of HRV, BPV and BRS, was not a sufficient cause to explain the occurrence of these hypotensive syndromes. An explanation for our findings might lie in the fact that hypotensive syndromes, especially in elderly patients, are multifactorial in genesis [36-38]. The lack of an association between HRV, BPV and BRS and hypotensive syndromes does not prove that cardiovascular autonomic control has no role in the pathophysiology of these hypotensive syndromes. However, it is unlikely that cardiovascular autonomic dysfunction is a sufficient factor, as other redundant regulatory mechanisms are able to compensate for diminished cardiovascular autonomic function and thus determine if hypotensive syndromes occur. Cardiovascular autonomic dysfunction probably is just a component cause with high frequency in geriatric patients and therefore there is no difference to be found between these groups. The conflicting data from literature can easily be explained by the geriatric syndrome causative model, which can be filled with different combinations of factors in different subgroups [36-39].

Strengths and Limitations

This study has some limitations. Firstly, this study was a cross-sectional analysis. Secondly, we have no complete assessment of all 14 different measures of autonomic function in all 203 patients. However, for 10 measures, there is approximately 15% or less missing data, which is acceptable. Of the four variables, the percentage of missing data was higher because for frequency analysis consecutive data series of 50-100 s are necessary which was sometimes not possible because the BP measurement was interrupted by calibrations, and ECG signals were sometimes disturbed through artefacts. Moreover, the calculation of the BRS with the spectral method requires that the coherence is more than 0.5, which explains the high portion of missing data for BRS-LF and BRS-HF. Data sets of 50–100 s are rather short to perform frequency domain analysis in which it is advisable to have data set lengths of at least 128 consecutive beats [40]. Hereby, the estimation especially of the LF measures might be less accurate. However, despite these shortcomings, all the results of the frequency domain

analysis are in line with the other determined variables of HRV, BPV and BRS.

Thirdly, this study investigated a selective patient population and did not have a control group. All patients were referred to an out-patient geriatric falls and syncope clinic, because of syncope, falls and/or dizziness. This has the disadvantage of a specific selection and therefore our sample is not a representative for the general population, but only for populations referred to similar outpatient clinics. On the other hand, this is the geriatric population with the highest relevance of hypotensive syndromes.

Finally, there is evidence that postural and postprandial BP responses vary during the day and therefore should be measured more than once [12, 31, 41]. It would be possible that we falsely classified patients to be without OH, PPH, or CSH, who might in fact have the same degree of autonomic dysfunction as those patients with OH, PPH, or CSH. We found a high prevalence of syndromes, comparable to other studies, which reduces the likelihood that we missed patients. Also, when comparing patients with and without syncope or falls, there still was no difference in autonomic function. Misclassification is therefore less likely.

This study has several other strengths. Firstly, the BP measurements and tests in our study were performed under standardized circumstances, with a beat-to-beat finometer (FinaPres), which is an accurate way to measure BP variability [20].

Secondly, we limited as much as possible the confounding effects of medication on our assessment of hypotensive syndromes and on our estimates of autonomic function. To do this, all medication was withheld from midnight the night before the tests. Nonetheless, biological effects of these drugs might persist longer than the period of abstinence, especially for beta-blockers. However, in aging beta-adrenoreceptor responsiveness decreases by several mechanisms and the effects of beta-blockers are therefore less pronounced [42]. Moreover, we found no differences in the use of beta-blockers or other medication between patients with and without hypotensive syndromes, with exception of lower use of angiotensin -converting enzyme inhibitors and angiotensin-II receptor antagonists in patients with CSH.

Thirdly, a strength of this study is that we investigated all three hypotensive syndromes in the same patients, where other studies only looked at one type of hypotensive syndrome [8, 26-34].

Finally, we investigated a rather large group of 203 geriatric patients on the presence of three common hypotensive syndromes, and this study addresses a potential mechanism underlying these common hypotensive syndromes.

CONCLUSIONS

In geriatric patients with hypotensive syndromes, cardiovascular autonomic function as measured by HRV, BPV and BRS is comparable to patients without such syndromes. These findings may favour a multifactorial pathophysiology of geriatric hypotensive syndromes and argue against a single or dominant etiological factor, that is, cardiac autonomic dysfunction. For clinical practice, our data underline that in the initial diagnostic work-up of elderly patients with falls, dizziness and syncope should follow the structured approach as proposed in the evidence-based syncope guidelines, which recognizes a multifactorial aetiology [13, 43].

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Chapter 11.

Dynamic cerebral autoregulatory performance during endotoxemia-induced systemic inflammation in humans

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ABSTRACT

Background

238 Sepsis, the systemic host response to a severe bacterial infection, is often complicated by brain dysfunction. Many indicators, such as reduced global perfusion, disruption of the blood-brain barrier and cerebral edema, point towards a link between cerebral perfusion and brain dysfunction in sepsis. An important factor in this relationship may be cerebral autoregulation.

The endotoxemia model has been used to study inflammatory-induced changes in cerebral autoregulation. Surprisingly, in contrast to observations in septic patients, an improved cerebral autoregulatory performance was observed during endotoxemia at 3-4 hrs after LPS infusion. This suggests that cerebral autoregulatory performance may change over time, with improvement at an early stage, but possible deterioration at a later stage. In the present study we investigated these temporal changes in dynamic cerebral autoregulation in healthy volunteers during experimental endotoxemia.

Method

Cerebral autoregulation was tested using transcranial Doppler ultrasound in healthy volunteers (n = 16) before and at several time points after LPS infusion (-2, 2, 4, 6 and 8 hours). Correlation coefficient analysis and transfer function analysis were used to quantify cerebral autoregulation.

Results

All subjects showed a marked systemic inflammatory response similar to that of the very early stages of sepsis, with progressive fever, leukocytosis and elevated TNF-α and IL-6 levels, after LPS infusion. In contrast to the hypothesis, no deterioration of the cerebral autoregulatory performance was found after LPS infusion.

Conclusion

Endotoxemia-induced systemic inflammation is associated with an overall preserved cerebral autoregulatory performance at an early stage after LPS infusion, with no deterioration up to 8 hrs.

INTRODUCTION

Sepsis, the systemic host response to a severe bacterial infection, is a potentially deadly medical condition, characterized by a widespread state of inflammation, often complicated by organ dysfunction or failure. Potentially irreversible acute cerebral dysfunction occurs in approximately 70 % of septic patients and may manifest as sepsis-associated delirium [1-3]. These changes in cerebral functioning are associated with increased rates of persistent cognitive dysfunction [4] and increased mortality rates after hospital discharge [5].

Despite the fact that the exact mechanisms of sepsis-induced encephalopathy remain obscure, many indicators, such as reduced global perfusion, disruption of the blood-brain barrier and cerebral edema, point towards a link between cerebral perfusion and brain dysfunction in sepsis [6, 7]. An important factor in this relationship may be cerebral autoregulation. Cerebral autoregulation refers to the physiological mechanism that maintains an adequate cerebral blood flow during changes in blood pressure. As blood pressure drops may frequently occur during the course of sepsis, disturbed cerebral autoregulation likely causes the brain to be excessively sensitive to hypoperfusion, resulting in ischemic damage. The few studies performed during sepsis have shown that cerebral autoregulation is indeed impaired in the great majority of patients and is associated with delirium [8, 9]. However, these observational association studies in patients lack the possibility to directly relate inflammation to the observed changes in cerebral autoregulation from a baseline situation.

Experimental human endotoxemia is an established human *in vivo* model of the systemic inflammatory response that occurs during early sepsis, including changes in renal function [10], vascular function [11, 12], as well as cerebral perfusion [8, 13]. In this model a low dose of purified *E.coli* lipopolysaccharide (LPS) is administered intravenously to healthy volunteers. LPS is one of the principal bacterial components that interact with the host during Gram-negative sepsis [14]. The endotoxemia model has been used to study inflammation-induced changes in cerebral autoregulation [8, 13]. Surprisingly, in contrast to observations in septic patients, an improved cerebral autoregulatory performance was observed during endotoxemia at 3-4 hrs after LPS infusion [8, 13]. This suggests that cerebral autoregulatory performance may change over time, with improvement at an early stage, but possible deterioration at a later stage. In the present study we investigated these temporal changes in dynamic cerebral autoregulation in healthy volunteers during experimental endotoxemia.

METHOD

Design and subjects

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This study was part of a larger trial that was registered under trial number NCT01522794. The study protocol was approved by the Ethics Committee of Radboud university medical center and complied with the Declaration of Helsinki including current revisions and the Good Clinical Practice guidelines. Sixteen healthy men, aged 21 ± 2 (mean ± standard deviation) years, participated in this study after providing written informed consent. Screening of the subjects within 14 days before the test revealed no abnormalities in medical history and/or physical examination. Routine laboratory tests and electrocardiogram were normal.

Subjects received a 20-gauge arterial catheter (Angiomat, Becton Dickinson, Sandy, UT, USA) for continuous intra-arterial blood pressure monitoring and blood sampling. Hemodynamic data were continuously monitored for safety. A venous cannula was placed to permit infusion of 1.5 L 2.5 % glucose/ 0.45% NaCl in one hour preceding LPS infusion (prehydration), followed by 150 mL/h until 6 h after LPS infusion and 75 mL/h until the end of the experiment to ensure an optimal hydration status [15]. At T=0 h subjects received a single intravenous dose of 2 ng/kg purified E. Coli LPS (Escherichia coli O:113, Clinical Center Reference Endotoxin, National Institute of Health (NIH), Bethesda, MD, USA) by slow injection administered over 1 min. The LPS infusion was followed by infusion of 5 ml isotonic saline to ensure complete delivery.

Dynamic cerebral autoregulation measurements

Dynamic cerebral autoregulation was assessed in the time domain using correlation coefficient analysis and in the frequency domain using transfer function analysis [16, 17], two widely used methods to quantify cerebral autoregulation from spontaneous fluctuations in blood pressure and cerebral blood flow velocity.

Cerebral blood flow velocity was estimated bilaterally in the middle cerebral arteries (MCA) by transcranial Doppler ultrasonography at time points: 2 h prior to LPS administration and 2, 4, 6 and 8 h after LPS administration. The left and right MCA were insonated by placing a 2-MHz Doppler probe (Multi-Dop, Compumedics DWL, Germany) over the temporal bone. The MCA were identified according to their signal depth, velocity, and wave characteristics [18]. After identification of the MCA, Doppler probes were locked at a constant angle and

position during data collection with a customized headband (Spencer technologies, Seattle, Wa, USA). If only one signal was available due to one-sided temporal window failure, we included this available signal for analysis. At each time point five minutes of beat-to-beat changes in mean blood pressure and cerebral blood flow velocity were obtained synchronically using a 4th order low-pass Butterworth filter. The consecutive beat-to-beat data of blood pressure and cerebral blood flow velocity were resampled at 10 Hz to create equidistant data sampling over time for spectral analysis.

Correlation coefficient analysis

Correlation coefficient analysis was performed in accordance with several investigations that showed that fluctuations on long time scale in cerebral blood flow velocity correlated with fluctuations in blood pressure [19, 20]. First, mean blood pressure and cerebral blood flow velocity were averaged over 3-second periods. Then, 20 consecutive 3-second averages of mean blood pressure and cerebral blood flow velocity were used to calculate single Pearson's correlation coefficients. Finally, the collected 5-minute mean correlation coefficients were averaged over the whole measurement period and labeled as the autoregulatory index.

Transfer function analysis

The transfer function analysis assumes that autoregulation mechanisms can be described by a linear dynamic system with blood pressure as input and cerebral blood flow velocity as output [17, 21]. Cerebral autoregulation was evaluated using the gain, phase and coherence obtained by transfer function analysis at the respective time points: 2 hours prior to LPS administration and 2, 4, 6 and 8 hours after LPS administration. Transfer function gain, phase and coherence were estimated using the cross-spectral method which has been described in detail previously [17, 21]. The time series of mean blood pressure and cerebral blood flow velocity were first subdivided into 950-point segments with 50 % overlap for spectral estimation. This process resulted in five segments of data for the segment periodogram average. Fast Fourier transforms were implemented with each Hanning-windowed segment and averaged to quantify the transfer function. The coherence spectrum is defined as the normalized modulus of the cross-spectrum; the phase spectrum is the argument of the (complex-valued) cross-spectrum; and the gain is the regression coefficient of cerebral blood flow velocity on blood pressure.

The transfer function parameter gain quantifies the damping effect between blood pressure

and cerebral blood flow velocity. A low gain indicates an efficient autoregulation, whereas an increase in gain represents a diminished efficiency of the dynamic process of cerebral autoregulation. The phase shift represents the time lag between changes in blood pressure and cerebral blood flow velocity. A larger phase shift (i.e. cerebral blood flow velocity oscillations precede blood pressure oscillations) indicates better cerebral autoregulation. Finally, coherence reflects whether the changes in cerebral blood flow velocity can be linearly explained by the changes in blood pressure. To ensure reliable TFA outcomes, a cut-off value of 0.5 for coherence was used. For quantification of cerebral autoregulation the positive phase shift and the gain between blood pressure and cerebral blood flow velocity were quantified as means of the following frequency bands: very low frequency (VLF): 0.02-0.07Hz; low frequency (LF): 0.07-0.15Hz; high frequency (HF): 0.15-0.4Hz [17, 21].

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Laboratory tests

EDTA anti-coagulated blood for the determination of tumor necrosis factor alpha (TNF-a), interleukin-6 (IL-6), IL-10, IL-1ra and C-reactive protein (CRP), was drawn one hour before endotoxin administration, and at t=0, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours afterwards. Blood samples were immediately centrifuged at 2.000 g for 10 minutes at 4°C and supernatants were stored at -80°C and until batch wise determination of cytokine concentrations using a simultaneous Luminex assay according to the manufacturer's instructions (Bio-plex cytokine assay; Bio-Rad, Hercules, California, USA).

STATISTICAL ANALYSIS

Statistical analysis was performed using PASW Statistics software version 18.0 (SPSS Inc., Chicago, IL, USA). Comparisons between T = -2 hours and the different time sets (2, 4, 6 and 8 hours) after LPS dosing were performed using mixed model analysis. Statistical significance was set at $p<0.05$. Data are presented as mean and standard deviation.

RESULTS

Subject characteristics

Figure 1 shows the systemic inflammatory response and the cardiovascular parameters of the subjects after LPS infusion. The injection of LPS caused flu-like symptoms including fever, which peaked at 4 h after LPS infusion (increase of 1.5 ± 0.5 ˚C), and

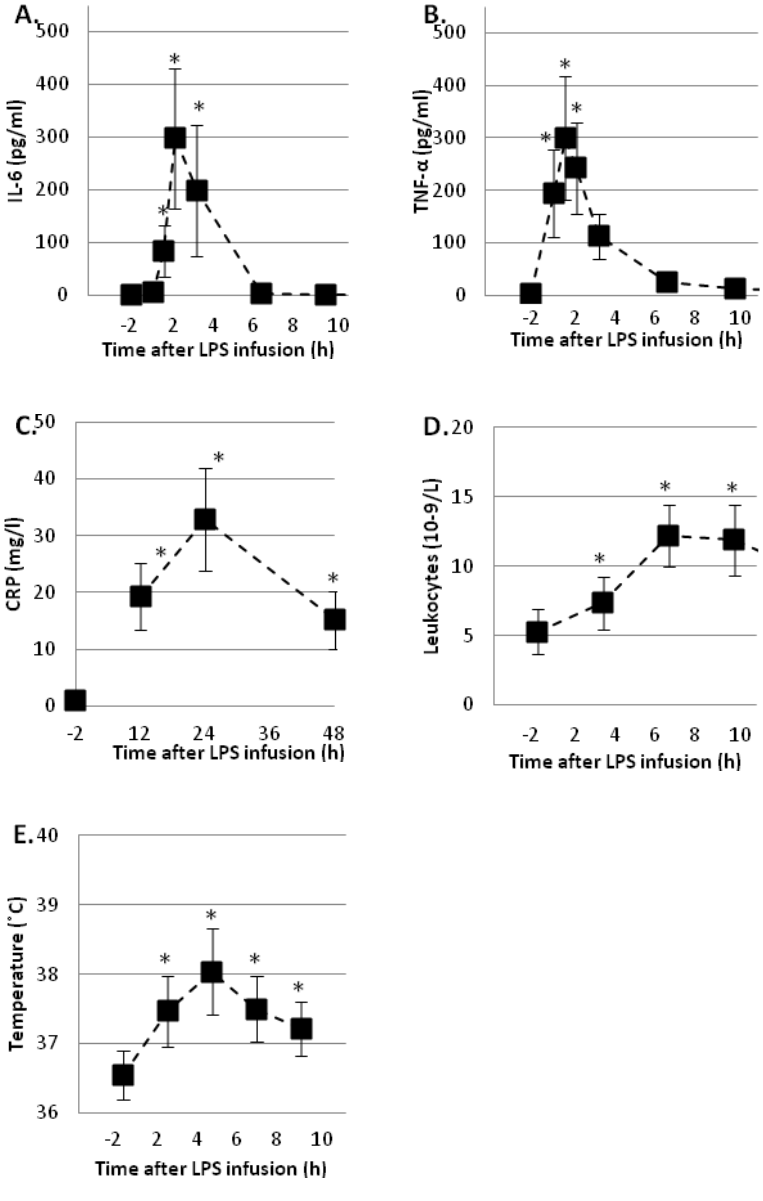

Figure 1. Systemic inflammatory response and cardiovascular parameters of the subjects after LPS infusion. X-axes show time after LPS infusion in hours. Y-axes represent for the graphs A to J: IL-6, TNF-α, CRP, leukocytes count, temperature, pCO2, pO2, heart rate, mean blood pressure, mean cerebral blood flow velocity, successively. Stars indicate a significant difference with respect to 2 hours before LPS infusion.

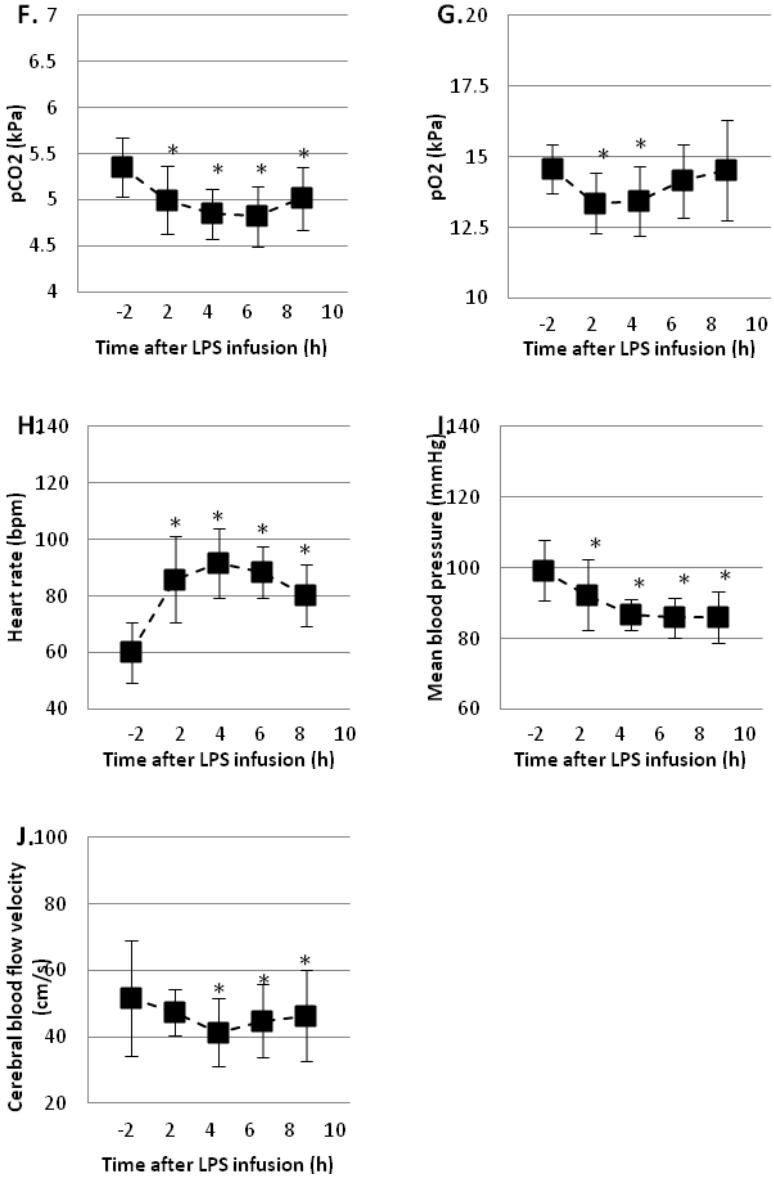

Figure 1. Continued

leucocytosis (13 ± 2 x109/L). All subjects had increases in plasma TNF-α, IL-6, IL-10 and IL-1RA. pCO_2 and pO_2 decreased after LPS infusion. It was not always possible to record cerebral blood flow velocity, due to discomfort caused by the Doppler headband in combination with the severity of the endotoxemia-associated symptoms. Recordings of blood pressure and cerebral blood flow velocity were completed in 14, 11, 15, 14 and 14 subjects at the time sets -2, 2, 4, 6 and 8 hours after LPS administration, respectively. Heart rate was maximal at T = 4 to 6 hours (increase of 33 ± 9 bpm). Mean blood pressure and mean cerebral blood flow velocity decreased after LPS infusion with a minimum around 6 hours after LPS infusion (decrease of 16 ± 7 mmHg and 16 ± 15 cm/s, respectively).

Correlation coefficient analysis

Pearson's correlation coefficients were calculated for the different time sets (-2, 2, 4, 6 and 8 hours after LPS dosing). Figure 2 illustrates the average group results of the correlation coefficient analysis. The correlation coefficient decreased at t = 4, representing a lower

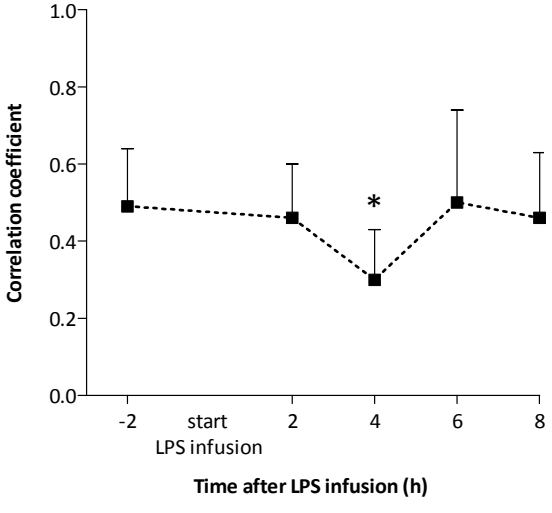

Figure 2. Outcomes of the correlation coefficient analysis in mean ± standard deviation of the subjects for -2, 2, 4, 6, and 8 hours after LPS infusion. Stars indicate a significant difference with respect to 0 hours after LPS infusion.

correlation between blood pressure and cerebral blood flow velocity indicating an improved cerebral autoregulation at 4 h after LPS infusion ($p < 0.01$). After 6 h after LPS infusion, the correlation coefficient did not differ from before LPS infusion anymore ($p>0.05$).

Transfer function analysis

Transfer function analysis was used to assess the cerebral autoregulation performance in the frequency domain. Figure 3 shows the results of the transfer function analysis after LPS infusion. Overall, results were consistent with the high-pass filter model of dynamic cerebral autoregulation (17) with highest phase values in VLF and decreases in phase from VLF to LF to HF, and lowest gain in VLF increasing from VLF to LF to HF. This pattern was present at all time points, suggesting preserved dynamic cerebral autoregulation. Within each frequency band (VLF, LF, HF), transfer function gain was stable over time. Phase showed more variation over time, but there were no significant reductions or increases over time in VLF or LF. Phase increased modestly in HF at 2 and 4 h ($p < 0.05$). No differences in transfer function metrics were found after 6 h after LPS infusion ($p > 0.05$).

DISCUSSION

This study confirms that controlled systemic inflammation in healthy volunteers is associated with overall preserved autoregulatory performance. In addition, serial measurements over time revealed that autoregulation was stable over time, with a possible short-term improvement 4 hours after endotoxin administration, whereas no deterioration could be found at a later stage.

Sepsis is defined by a systemic response to infection that includes fever, rapid heartbeat and respiration, and may result in hemodynamic instability and organ dysfunction. Acute brain dysfunction is often one of the first symptoms of organ dysfunction in septic patients and is associated with an unfavourable neurocognitive outcome [22]. An important precipitating factor for the neurocognitive decline may be a reduced cerebral blood flow due to a reduced capacity of cerebral autoregulation to compensate for the reduction in and instability of cerebral perfusion pressure (i.e. blood pressure).

Two previous studies have evaluated cerebral autoregulation in a human experimental model of the systemic inflammatory response [8, 13]. Both studies used transfer function analysis to investigate cerebral autoregulation in healthy volunteers before and after LPS infusion and

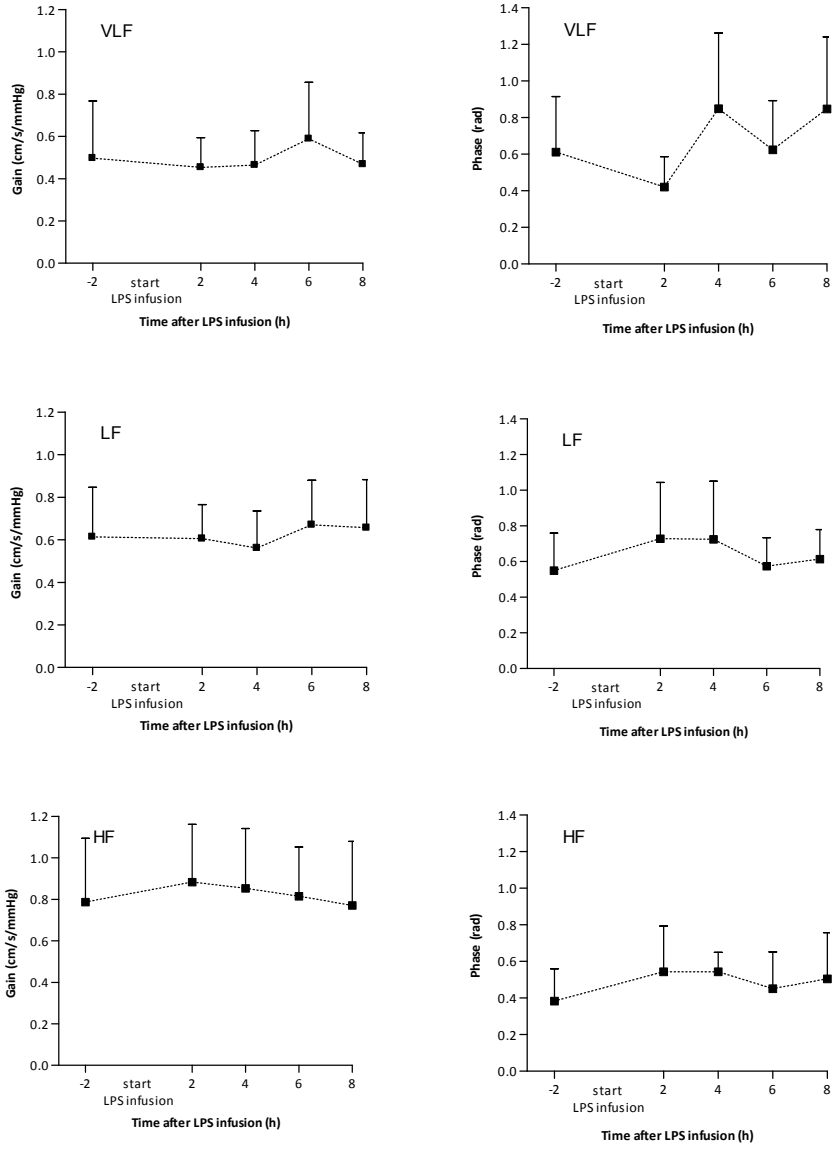

Figure 3. Transfer function results of the subjects for -2, 2, 4, 6, and 8 hours after LPS infusion in mean ± standard deviation. Stars indicate a significant difference with respect to 0 hours after LPS infusion.

concluded that cerebral autoregulation was enhanced at an early stage after LPS infusion (3 and 4 hours after LPS infusion). This conclusion was in contrast to the observed autoregulatory performances in septic patients where an impaired cerebral autoregulation
248 was found. Berg et al. speculated that one of the reasons that they found a difference in autoregulatory performance between patients and volunteers originates from the shortly lived inflammatory response during experimental endotoxemia. As it is possible that the autoregulatory performance improves at an early stage, but deteriorates at a later stage after LPS infusion, we studied the autoregulatory performance at several time points after LPS infusion, up to 8 hours after LPS infusion. In addition to the previous studies, we also expanded the methodology for the quantification of the cerebral autoregulatory performance by investigating the transfer function metrics over three frequency bands (VLF, LF and HF) instead of only using the LF band, and by using a second, frequently used method to analyze the data (correlation coefficient analysis).

Confirming the previous studies, we found an overall preserved cerebral autoregulation during systemic inflammation evoked by experimental endotoxemia. Importantly, we established that no deterioration of the cerebral autoregulatory performance occurred at a later stage. The findings of the previous [8, 13] and present study in healthy volunteers subjected to endotoxemia are in contrast with reports of impaired cerebral autoregulatory performance in septic patients [8, 23, 24]. An explanation for this discrepancy may be that, although the endotoxemia model has been shown to be a valuable model to study some aspects of sepsis, it may not be an appropriate model to study the changes in cerebral autoregulatory performance observed during sepsis. A number of physiological and pathophysiological mechanisms may be involved in the deterioration of autoregulatory performance during sepsis, such as a comprised circulatory state [25], use of medication that may interfere with autoregulatory function like vasopressors [26], or a changed oxygen extraction due to mitochondrial dysfunction [27]. Studies on the effect of the above mentioned mechanisms on cerebral autoregulatory performance are warranted to elucidate their role in the deterioration of the cerebral autoregulatory performance in septic patients, giving more information on the occurrence of cerebral dysfunction at various stages of the disease. In addition, there are methodological reasons that may suggest that cerebral autoregulatory performance is not relevantly impaired in patients with sepsis. To date only three studies are published investigating dynamic cerebral autoregulation in septic patients [8, 23, 24]. Pfister et al. and Steiner et al. used correlation coefficient analysis to quantify cerebral autoregulation [23, 24]. Impaired cerebral autoregulation was indicated as a correlation coefficient index >0.3 and >0, respectively. Using these cutoff values for

correlation coefficient analysis would indicate our healthy subjects to have impaired cerebral autoregulation at each time point, even before administration of endotoxin. Furthermore, studies have shown positive values for the correlation coefficient index in healthy subjects of, 0.2±0.13 [28], 0.28±0.18 [29], and 0.23±0.1 [30]. Berg et al. quantified dynamic cerebral autoregulation using transfer function analysis [8]. Comparing cerebral autoregulatory functioning in septic patients with healthy volunteers, they found a maintained transfer function gain, albeit a lower transfer function phase, concluding an impaired autoregulatory functioning. However, they only included 6 patients in their transfer function analysis. Furthermore, they mention that the spectral power of the blood pressure was very low for the whole group of patients. Despite the fact that they left out those subjects with a transfer function coherence value below 0.4, this low variation in blood pressure make the transfer function results less reliable. These results indicate that transfer function analysis may not be an appropriate technique to quantify cerebral autoregulation in septic patients.

The relative stability in the parameters of dynamic cerebral autoregulation were observed despite marked changes in blood pressure, heart rate, cerebral blood flow-velocity and end-tidal pCO_2. Mean blood pressure decreased with 13 mmHg (-13 %) between -2 and 4 h following LPS. Cerebral blood flow velocity was not stable, but decreased, despite absent recovery in blood pressure. This decline in cerebral blood flow velocity could suggest impaired static autoregulation. However, this interpretation would require stable pCO_2 levels, which was not the case. Changes in CO_2 strongly affect cerebral blood flow and the 0.5 kPa reduction in end-tidal CO_2 may explain the 15 % reduction in CBF [31]. With prolonged changes in CO_2, adaptation occurs however and cerebral blood flow may partially restore towards baseline. The reduction in $etCO_2$ may also explain the finding of enhanced cerebral autoregulation with correlation analysis. Studies in healthy controls have shown augmented dynamic cerebral autoregulation with hypocapnia [32].

There are several limitations to the present study. First, the inflammatory insult may be too short and to mild to cause a deterioration of cerebral autoregulation. Endotoxin is administrated as bolus infusion, which results in a marked, but short-lived inflammatory response. Second, because of the relatively small study group, these preliminary results would need to be confirmed in a larger group of subjects, where the LPS dose should be administered as a continuous infusion over a few hours, rather than bolus administration. Third, the methodology used to quantify cerebral autoregulation is not sensitive enough to measure the changes in autoregulatory performance that occur after LPS infusion. Currently, no generally accepted gold standard test for cerebral autoregulation exists. Both correlation

coefficient analysis as transfer function analysis are based on the assumption of a linear relationship between blood pressure and cerebral blood flow velocity. As, in general, cerebral autoregulation is considered to be a non-linear phenomenon, these methods possibly produce misleading results when nonlinear properties are present. However, transfer function analysis has shown changes in cerebral autoregulation metrics in patients with sepsis.

In conclusion we demonstrate that endotoxemia-induced systemic inflammation is associated with an overall preserved, possibly even enhanced, cerebral autoregulatory performance at an early stage after LPS infusion, with no deterioration up to 8 h.

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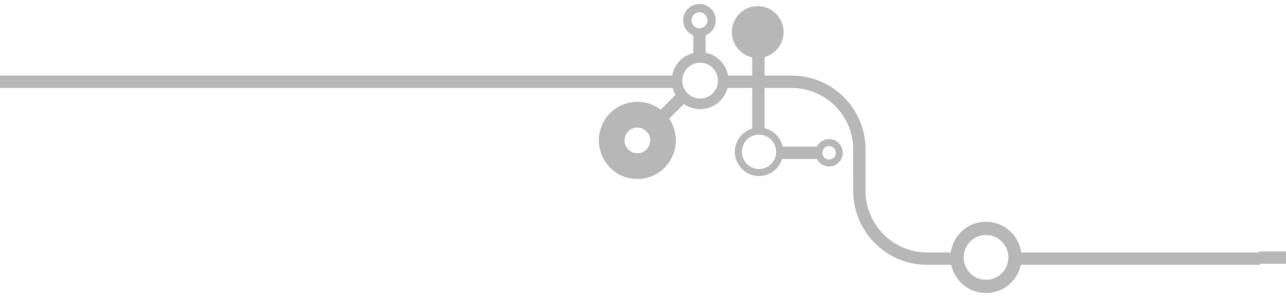

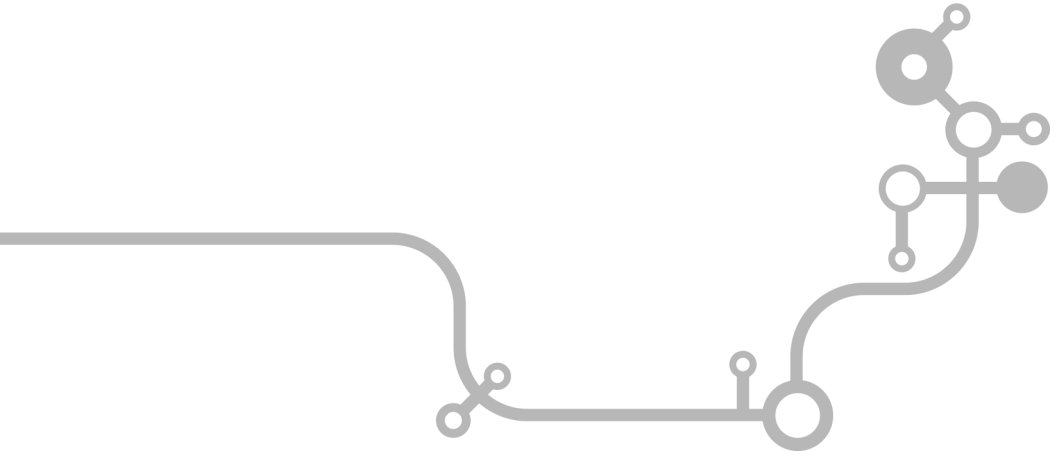

Chapter 12.

General discussion

Over the past years, the interest in the effects of diseases on haemodynamics and vice versa the effect of changes in haemodynamics on the pathogenesis of diseases has been growing. This has led to important progress in techniques to estimate cerebral perfusion and analytical methods to evaluate regulation systems, such as baroreflex functioning and cerebral autoregulation. Worldwide, researchers from several disciplines, i.e. medicine, physiology, engineering and physics, are trying to get a grip on the haemodynamic regulation systems of the human body. A variety of measurement techniques are being employed in clinical and experimental settings. New publications with novel findings rapidly follow one another.

Despite considerable scientific interest, currently a standardized test to assess cerebral haemodynamics is still lacking. This is one of the reasons why the monitoring of cerebral autoregulation is not yet included in clinical routines. Being able to measure and monitor cerebral autoregulation in a sensitive and robust way would provide clinically useful information and would permit a more individualized physiologically based therapy aimed at reducing the risk of secondary brain injury. The studies conducted in this thesis are part of the active, ongoing scientific debate on the best measurement technique for cerebral autoregulation. The aim of this thesis is to gain better insight in the quantification of cerebral autoregulation and how the perfusion regulation is affected in different pathophysiological conditions. The main research questions are:

1) How is transfer function analysis applied for the quantification of cerebral autoregulation?

2) Is the perfusion regulation impaired in patients with Alzheimer's disease, frail elderly and/ or during systemic inflammation?

With the outcomes of this thesis, we hope to stimulate the scientific community to step towards uniform use of quantification techniques for cerebral autoregulation. This chapter starts with the main findings of this thesis. Then the findings are discussed, ending with conclusions and future perspectives.

MAIN FINDINGS

The first main research question of this thesis is: **How is transfer function analysis applied for the quantification of cerebral autoregulation?** Below, the main findings are summarized.

- A strong diversity exists in the application of transfer function analysis. No standard protocol exists and most studies fail to report important settings that were used to calculate the transfer function. (Chapter 2)

- When the transfer function results of different studies are pooled, despite the strong methodological diversity, the data still show the high pass filter behaviour for dynamic cerebral autoregulation. (Chapter 2)

- When cerebral autoregulatory performance is quantified from the same dataset, but by different methods of analysis as used in different centres, metric outcomes show a large variability. (Chapter 3)

- In contrast to the analysis of sit-stand manoeuvres and cerebral vasomotor reactivity tests, transfer function analysis seems less sensitive to detect subtle impairment in cerebral autoregulatory functioning in small populations. (Chapter 6)

- Standardisation and validation of the quantification method for cerebral autoregulation is needed to improve the reliability and usefulness of cerebral autoregulation in clinical practice. (Chapter 2 and 3)

- Transfer function settings and criteria for qualifying terms such as 'impaired cerebral autoregulation' should be defined to make comparison between different studies possible. (Chapter 3 and 4)

- Haemodynamic analysis at a group level may already provide clinical useful information and is applicable in a variety of populations. (Chapter 6, 7, 8, 9, 10 and 11)

The second main research question of this thesis is: **Is the perfusion regulation reduced in patients with Alzheimer's disease, frail elderly and/or during systemic inflammation**? Hereafter the results for this research question are given.

- Patients with Alzheimer's disease have an impaired cerebral autoregulation, cerebral vasomotor reactivity and baroreflex functioning. This might cause chronic mild hypoperfusion, which may contribute to the brain atrophy seen in Alzheimer's disease. (Chapter 6 and 7)

- Aging is accompanied by a decline in slow oscillations of cerebral haemodynamics and blood pressure, possibly due to less spontaneous activity in microvascular smooth muscle cells and increased vessel stiffness. (Chapter 8)

- In geriatric patients with hypotensive syndromes cardiovascular autonomic function is comparable to patients without such syndromes. (Chapter 10)

- Endotoxemia-induced systemic inflammation does not deteriorate the cerebral autoregulatory performance. (Chapter 11)

DISCUSSION

Quantification of cerebral autoregulation

The first part of this thesis deals with the research question: 'How is transfer function analysis applied for the quantification of cerebral autoregulation?'. Transfer function analysis is, currently, the most used non-invasive method for the quantification of cerebral autoregulation. The method is based on frequency analysis between spontaneous changes in blood pressure and cerebral blood flow velocity. Cerebral autoregulation is evaluated by dividing the cross spectra between blood pressure and cerebral blood flow velocity by the auto spectra of the blood pressure. To estimate these spectra several parameter settings need to be chosen, such as sample frequency, window length, overlap percentage and filtering. Together, these settings determine how the relationship between blood pressure and cerebral blood flow velocity will be approached. By performing a review of the literature (**Chapter 2**), we investigated how the transfer function analysis has been applied for the quantification of cerebral autoregulation, and the effect of different approaches of transfer function analysis was investigated in a multi-centre study (**Chapter 3**).

Overall, we found that, while at first sight transfer function seems straight-forward, the standardization of the methodology was weak. It was found that most studies fail to report important settings that were used to calculate the transfer function. This may be due to the fact that some of the investigators were not aware of the existence of different options for settings or did not see the need for mentioning them. However, the review did also not show any consistency in the well reported settings. Overall, the approach for transfer function analysis seemed a matter of personal preference of the investigators; a high diversity existed and no standardization could be found. This was an important observation as it was shown, with the multi-centre study described in **Chapter 3**, that the outcomes for cerebral autoregulation of the transfer function analysis do depend on the choice of transfer function settings. In this multi-centre study, the same dataset was analyzed by 15 different centres, using their own methodological approach for transfer function analysis. And although the same dataset was analyzed, a high diversity in outcome values was found between the centres.

From this, we can conclude that interpretation of the transfer function outcomes for cerebral autoregulation can only be done with care, taking into account the parameter settings used for the quantification. As many studies presented in literature have not well documented

their transfer function approach, one should also be careful when comparing study results.

Despite all this, we still believe that transfer function analysis may be of potential use in clinical research, since also encouraging results were found. First, in spite of a high variability, it was also shown in **Chapter 2** that combining the results of different studies does reveal multi-centre frequency plot trends for phase and gain that are consistent with the original high pass filter model for cerebral autoregulation. This suggests that, even when transfer function analysis is approached differently, the same physiological phenomenon is being investigated by the different centres. Furthermore, the multi-centre study showed that most centres were able to distinguish between normal cerebral autoregulation and modified cerebral autoregulation (using hypercapnia). Even the poorest performing centre had a ROC AUC higher than 0.75.

However, as the effect of many transfer function settings on the outcome for cerebral autoregulation is still unclear, we believe that standardization of the methodology is necessary to reduce inter-centre variability and to make comparisons between studies possible. A first step to standardization is set by the proposed guideline for the usage of transfer function analysis for the quantification of cerebral autoregulation described in **Chapter 4**. This preliminary guidelines is set up for the purpose of uniformity in use of transfer function analysis and future revisions in the light of new evidence may be necessary. Furthermore, it needs to be emphasized that this guideline does not intend to endorse transfer function analysis as the 'best' method for the quantification of cerebral autoregulation. Many other techniques exist which may be just as good or even better. Below some methodological considerations of transfer function analysis will be discussed, which certainly should be kept in mind when transfer function analysis is being employed.

First, transfer function analysis requires relatively long recordings (5 minutes or more) of blood pressure and cerebral blood flow velocity [1, 2]. This can be quite difficult in, for example, unstable patients.

Secondly, transfer function analysis between blood pressure and cerebral blood flow velocity is only reliable when the blood pressure contains sufficient variation. In the previous mentioned studies, we only used recordings of spontaneous blood pressure and cerebral blood flow velocity. These spontaneous blood pressure variations have the advantage not to depend on active patient cooperation or physiological interference through for example medication. More powerful oscillations, i.e. provoked by sit-stand manoeuvres [3], may enhance clinical significance as they further challenge cerebral autoregulation functioning.

Substantial short‐term manipulations of blood pressure, are, however, not suitable in cases of severe illness.

Third, transfer function analysis is based on the assumption of a linear relationship between blood pressure and cerebral blood flow velocity as it models a linear input-output system with the blood pressure signal as the input and the cerebral blood flow velocity signal as the output. In general, cerebral autoregulation is considered to be a non-linear phenomenon because of the nonlinear responses of key physiologic parameters [4], such as cerebrovascular resistance and arteriolar diameter, to changes in blood pressure [5]. Despite that the assumption of linearity may be justified in the case of spontaneous changes in blood pressure, because these changes are relatively small [6], transfer function analysis has the potential to produce misleading results in a system with nonlinear properties. It is arguable that a non-linear model may be more appropriate for assessment of autoregulation [7]. We described a new non-linear analysis method, convergent cross mapping, to assess these nonlinear dynamics of cerebral autoregulation.

Furthermore, it is questionable whether, even when non-linear effects are left out, it is accurate to assume that transfer function analysis wholly reflects cerebral autoregulation. Although the relationship between blood pressure and cerebral blood flow is influenced by dynamic cerebral autoregulation, it is important to recognise that other physiological factors such as baseline blood pressure, pCO_2 [8, 9], brain metabolic activity, and sympathetic tone [10] are also influential. Because transfer function analysis considers only the blood pressure (input) and cerebral blood flow (output) relationship, the approach is clearly a simplification of a highly complex physiological system.

The last methodological issue that will be discussed is that the settings used for transfer function analysis possibly depend on the signals used as input. For our review and multi-centre study we focused on using cerebral blood flow velocity measured with transcranial Doppler. Although transcranial Doppler offers the temporal resolution necessary to study dynamic cerebral autoregulation, velocity is not necessary equal to flow. Changes in the measured flow are proportional to the changes in mean velocity only if the diameter of the middle cerebral artery remains constant. Numerous studies have shown that middle cerebral artery diameter in humans remains relatively constant under a variety of haemodynamic conditions [11-13]. However, small changes in diameter may cause large variations in velocity, leading to a higher spread in transfer function outcomes. Other surrogates, i.e. obtained with near-infrared spectroscopy (Chapter 8), may provide better estimates of cerebral blood flow, however when used, the settings for transfer function analysis should possible be adopted.

Clinical application of haemodynamic analysis

From the previous paragraph it is clear that methodological improvements have to be made before quantification of cerebral autoregulation as a clinical test in an individual is possible. In the second part of this thesis, haemodynamics were evaluated in different patient groups. The chapters in this part of the thesis show that, in the current state, haemodynamic analysis at a group level may already provide clinical useful information. Furthermore, it shows that haemodynamic analysis is applicable in a variety of populations, such as in the very old and critically ill. In this section, the clinical applications of haemodynamic analysis, as it was performed in this thesis, will be discussed.

Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder and is the leading cause of dementia. At this moment, there is still limited understanding of this disease and its underlying cause, which is reflected in the lack of an effective curative treatment.

A growing body of evidence suggests a strong link between Alzheimer's disease and affected vascular function. However, the exact relationship between vascular functioning and Alzheimer's disease remains poorly understood. We investigated whether cerebral autoregulation (**Chapter 6**), cerebral vasomotor reactivity (**Chapter 6**) and baroreflex functioning (**Chapter 7**) are impaired in patients with Alzheimer's disease.

Momentarily, three studies have investigated dynamic cerebral autoregulation in patients with Alzheimer's disease [14-16], but found no evidence for impairment of cerebral autoregulatory performance. These studies assessed cerebral autoregulation using transfer function analysis. However, as shown in the first part of this thesis, this method may be less sensitive to detect more subtle impairment in cerebral autoregulatory performance in small samples. Therefore, in our study, next to transfer function analysis to assess the cerebral autoregulatory performance, subjects were asked to perform a single sit-to-stand protocol and repeated sit-stand manoeuvres. These sit-stand manoeuvres provide a straight-forward measure for cerebral autoregulation. Changes in cerebral blood flow velocity and cerebrovascular resistance are investigated during reductions and increases in blood pressure without the necessity of a pharmacological intervention. Analysis of the manoeuvres did indeed show less effective damping by cerebral autoregulation.

Cerebral vasomotor reactivity was evaluated by studying changes in cerebral blood flow velocity, blood pressure and cerebrovascular conductance index during transitions from hypocapnia (induced by 30 s of coached hyperventilation) to normocapnia and from normocapnia to hypercapnia (induced by the inhalation of a gas mixture containing 7% CO_2). Like two previous studies, we have found an impaired cerebral vasomotor reactivity in Alzheimer patients.

Baroreflex functioning was quantified using a bivariate causal model (ARXAR model), which was first introduced by Nollo et al. (2001) [17]. In contrast to widely used non-invasive methods to quantify baroreflex functioning, such as heart rate variability and systolic blood pressure variability, this method has the advantage of describing the causal relationship from systolic blood pressure to the beat-to-beat interval of the heart rate and separates it from the mechanical pathway (from R-R interval to systolic blood pressure). The method revealed distinct lowered baroreflex functioning in patients with Alzheimer's disease compared with age-matched control subjects. In addition, the performance of the baroreflex functioning in the studied subjects with mild cognitive impairment was in between that of the Alzheimer patients and the controls. Moreover, we were able to confirm both these observations in an independent validation sample. Despite these clear results, we have to keep in mind that this is the first report in which a bivariate causal model is used to estimate baroreflex functioning in patients with Alzheimer's disease. Therefore, further evidence must be obtained before the method can be widely used in clinical settings, i.e. against important differential diagnoses in memory clinics.

Overall, we have found impaired cerebral autoregulation, cerebral vasomotor reactivity and baroreflex functioning in patients with Alzheimer's disease. The diminished baroreflex functioning, which was also observed in the patients with mild cognitive impairment, may imply that early in Alzheimer's disease besides cognition also blood pressure regulation is affected. This affected blood pressure regulation may lead to higher fluctuations in blood pressure. As impaired cerebral autoregulation means that the ability to stabilize cerebral blood flow during blood pressure fluctuations is reduced, the results of these studies point towards a higher risk for hypo- and hyperperfusion in patients with Alzheimer's disease. Despite the fact that the observed impairments are subtle, and do not directly lead to severe hypoperfusion, and may thus not lead to cerebral ischemia, it is not unthinkable that chronic mild hypoperfusion does contribute to brain atrophy in Alzheimer's disease. It can be argued that the observed impairments are caused not by Alzheimer's disease but by comorbid vascular disorders that may be more prevalent in Alzheimer's disease. Indeed, even though

we tried to match for hypertension, the patients with Alzheimer's disease did have a higher blood pressure. Still we believe that hypertension alone does not explain our results, as the study described in **Chapter 9** reveals no distorted cerebral autoregulatory performance in patients with hypertension.

Frail elderly

Longevity increases worldwide. Currently, about 11 % of the Dutch population is aged 70 years or older and this number is expected to be doubled by 2014. Currently, cardiovascular diseases are the most common cause of death among elderly patients in the Western World and almost 70% of the population over the age of 70 has some degree of hypertension. Furthermore, medications such as beta blockers and angiotensin converting enzyme inhibitors are commonly used in this age group, and the resultant drug interactions may compromise haemodynamic stability and lead to untoward events. Understanding haemodynamic changes that accompany the aging process is therefore becoming increasingly relevant. In this part of the thesis, we have shown that it is possible to measure systemic and cerebral haemodynamics in (frail) elderly.

In **Chapter 8**, blood pressure and oxygenated- and deoxygenated haemoglobin concentrations were continuously measured in healthy elderly aged over 63 years to investigate age-related changes in blood pressure and cerebral haemodynamics. The oxygenated- and deoxygenated haemoglobin concentrations were measured using near-infrared spectroscopy. Near-infrared spectroscopy is a promising non-invasive alternative for transcranial Doppler. Like transcranial Doppler, near-infrared spectroscopy has, in contrast to CT, MRI and PET, a high temporal resolution. Therefore it is more suitable to measure dynamic changes. Near infrared spectroscopy provides continuous quantification of regional changes in oxygenated and deoxygenated haemoglobin in the outermost layers of the cerebral cortex. The technique is based on near-infrared light absorption changes that depend on concentration changes of the chromophores oxygenated and deoxygenated haemoglobin in the tissue under investigation. Changes in total haemoglobin, defined as the sum of the changes in oxygenated and deoxygenated haemoglobin, can be used as a cerebral blood flow surrogate. The main advantage of near-infrared spectroscopy compared to transcranial Doppler is that the sensors are placed on the forehead and do not require precise location. In a survey reviewing transcranial Doppler results from 60 laboratories in the United States, percentages for failure to measure cerebral blood flow velocity due to inadequate access of the temporal window ranged between 0% and 65% (mean, 16%) [18]. Furthermore,

studies have shown that with advancing age assessing cerebral blood flow velocity through the temporal window becomes more and more difficult. Therefore, near-infrared spectroscopy may be a promising alternative.

However, the difficulties with using near-infrared spectroscopy as a surrogate of cerebral blood flow are that blood oxygenation is influenced by multiple factors, such as cerebral metabolism, arterial saturation and haematrocrit and that the "normal range" has yet to be defined. Furthermore, this study showed that the slow oscillations in near-infrared spectroscopy metrics decline with aging, presumably due to aging-related changes in the microvasculature such as vessel stiffness. Effects of age should therefore be taken into account when studying non age-related effects in near-infrared spectroscopy data. Further studies on the applicability of near-infrared spectroscopy for investigating haemodynamics are warranted.

Chapter 9 presents a case report on three patients (1 man and 2 women) over the age of 70 years, with hypertension, before and after anti-hypertensive treatment. Anti-hypertensive treatment is believed to be harmful in elderly patients. As organs in hypertensive elderly are accustomed to perfusion at higher pressures, decreasing blood pressure toward the "low-normal range" of healthy adults may be detrimental. Studying changes in systemic and cerebral haemodynamics due to medication use is of great importance.

All three patients completed an extensive test protocol, consisting of a baseline measurement, a sit-stand protocol and a cerebrovascular motor reactivity test, during which blood pressure (using photoplethysmography), cerebral blood flow velocity (using transcranial Doppler) and oxygenated- and deoxygenated haemoglobin concentrations (using near-infrared spectroscopy) were measured.

In the three elderly patients with hypertension described in this case report it was shown that blood pressure lowering did not result in a lowering of the cerebral blood flow. In contrast, even a rise in cerebral blood flow was observed. Furthermore, cerebral autoregulatory functioning was preserved in all three patients.

Overall, the findings of this case report have clinical significance since they suggest that it is save to apply anti-hypertensive treatment in elderly patients, with the benefit of reducing the development of (new) coronary events, stroke and congestive heart failure. However, as these results are only obtained in a small sample of three patients, a larger observational trial

is needed to confirm our findings.

In **Chapter 10** cardiovascular functioning was tested in 203 patients who visited the geriatric outpatient falls and syncope clinic of the Radboud university medical centre. These patients belong to a frail population, with a history of falls, dizziness and syncope. Furthermore, 6% of the patients was also diagnosed with a form of dementia. Fourteen different non-invasive autonomic function indices were used for heart rate variability, blood pressure variability and baroreflex sensitivity. No differences were found in autonomic function indices between the patients with hypotensive syndromes and those without such syndromes. These results argue against autonomic dysfunction as the sole or dominant factor in the pathophysiology of these syndromes and underline the need for a multifactorial approach in diagnostic studies of elderly people with problems as falls and syncope.

Endotoxemia-induced systemic inflammation

Experimental human endotoxemia is a human in vivo model, which, at least partially, mimics the inflammatory response that occurs during early sepsis. It is a well established model, which has been extensively studied. In **Chapter 11**, 16 healthy young men received a bolus injection of *E. coli* lipopolysaccharide to study the effect of inflammation on cerebral autoregulatory performance. Blood pressure and cerebral blood flow velocity were measured at different time point, 2 hours before and 2, 4, 6 and 8 hours after administration of endotoxin. This study shows that it is even possible to measure haemodynamic functioning in critically ill patients. However, as the *E. coli* lipopolysaccharide caused severe endotoxemia-associated symptoms, such as fever, nausea, shivering, muscle and back pain and headache, it was not always possible to record the cerebral blood flow velocity and thus quantify cerebral autoregulatory performance. This was mainly due to discomfort caused by the Doppler headband, which could also be a problem in other clinical settings. Still, in contrast to conventional imaging modalities, such as MRI and CT, which require transferring patients to imaging facilities, transcranial Doppler is an easy to use, safe technique.

These study results point towards an overall preserved cerebral autoregulatory performance and two opposite conclusions could be drawn. First, it is possible that - in contrast to previous studies - the cerebral autoregulatory performance is not impaired in patients with sepsis and therefore not a cause of sepsis-associated cerebral dysfunction. Second, it could be that cerebral autoregulatory performance is impaired, but the endotoxemia model is not an appropriate model to study the changes in cerebral autoregulatory performance observed

during sepsis. Unfortunately, our study is inconclusive in this regard. As it is not ruled out that impaired cerebral autoregulatory functioning is one of the causes of sepsis-associated cerebral dysfunctioning, further studies on cerebral autoregulation in patients with sepsis are recommended.

PERSPECTIVES OF CEREBRAL AUTOREGULATORY TESTING

From the results of this thesis it is clear that cerebral autoregulatory testing may be of great clinical benefit. However, optimization of the methods to quantify cerebral autoregulation is needed before they can implemented in the clinical arena.

The first observation of cerebral autoregulation was described by Fog in 1937 [19]. Fog investigated the cat's pial vessels during manipulations of the blood pressure and observed that the pial autoregulatory vasomotor responses were independent of neurogenic stimuli. The concept of cerebral autoregulation in terms of blood flow constancy during perfusion pressure changes was finally established by Lassen in 1959 [20]. From then on there has been an increasing scientific and clinical interest in the quantification of cerebral autoregulation and the role that impairment of the cerebral autoregulatory functioning might play in the causation, progression and risk of disorders such as intracranial haemorrhage, stroke and neurodegenerative diseases.

Over the last 20 years, a wide variety of techniques has been developed and adopted for the assessment of cerebral autoregulation, but without providing a gold standard [6]. Transfer function analysis was first applied in 1990 for the quantification of cerebral autoregulation [21] and has been used in many different clinical populations ever since. However, the findings of this thesis show that the transfer function outcome is highly dependent on the application form. The lack of application consistency between research centres is clinically problematic because inappropriate conclusions may be drawn depending on the application chosen for the quantification of cerebral autoregulation. For example, when used in individual care, transfer function in one way may classify the individual as having normal cerebral autoregulatory functioning, while another application format will classify the patient as having deficient cerebral autoregulatory functioning.

Thus, the findings of this thesis clearly question whether it is appropriate to continue the use of transfer function analysis as a reflective of cerebral autoregulation, without knowing the exact effects of transfer function settings on outcome metrics. Therefore, before getting

transfer function analysis to the clinical arena, further experimental validation of the transfer function metrics is needed.

270 However, this does not necessarily invalidate previous research or preclude the future application of transfer function analysis. Transfer function analysis, in its various ways, may predict cerebral autoregulatory performance on a population level but provide nonspecific physiological information on an individual patient basis. This is also true for metrics as blood pressure and heart rate variability which are predictive of cardiovascular outcomes in large-scale clinical trials [22, 23], but their physiological basis is poorly understood [24, 25].

Overall, our findings highlight the need for better understanding of the physiological information that each way of application of transfer function analysis may or may not convey. Therefore, many steps have to be taken before transfer function analysis can be considered to represent a valid standard for the assessment of cerebral autoregulation in individuals in clinical practice. Until such time, it is important that researchers use uniform settings for their cerebral autoregulation studies to ascertain that variations found in outcome metrics between studies are not caused by methodological differences. In this thesis, a guideline is provided for uniform application of the transfer function analysis (**Chapter 4**). This guideline contains directions for the data acquisition, measurement protocol and application of transfer function analysis. While there might be arguments for not adopting the proposed settings, we believe that when researchers stick to these directives (even if only as additional results) reproducibility and implementation of study results will improve.

We emphasize that currently the evidence in support of some of the proposed settings is still weak, therefore arguments against the use of the current guideline and new insights from future research should be used in order to shape and improve the guideline. This is an important ongoing process, as until a global consensus is reached transfer function analysis can only be applied as a scientific tool to predict outcome on a population level and physiological interpretation of past and future research using transfer function analysis should be done with caution.

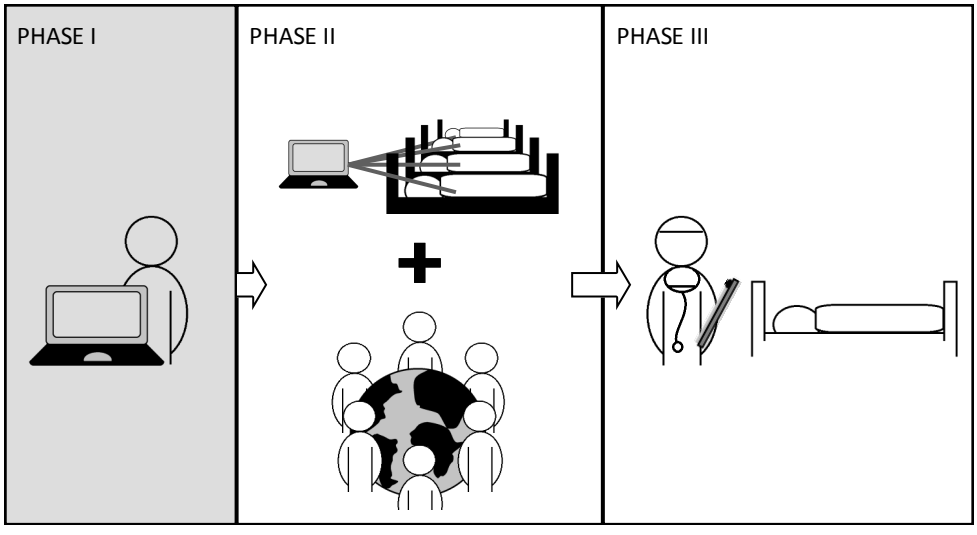

Figure 1.

At this moment, the cerebral autoregulatory research is still in its first stage, in which different methods are tested to investigate whether they can be used as reflective of the cerebral autoregulatory functioning. Before, cerebral autoregulatory testing can finally be implemented in the clinical arena, further experimental validation of the methods is needed. The methods should be tested in several clinical populations to investigate their sensitivity, specificity and robustness. And a global consensus should be reached on the application of the method and the interpretation of the method outcomes.

CONCLUSION

Monitoring cerebral autoregulatory performance can help in the early detection of an increased risk of brain damage, due to hypo- or hyperperfusion. Many situations exist in which a repeatable and safe method to assess the autoregulatory capacity would be useful, such as in the care of patients with head injury, suffering from sepsis, intracranial haemorrhage or stroke, but it can also play a role in early detection of neurodegenerative diseases. However, due to the lack of standardization no normative values exist and therefore comparison between study results of different centres is difficult. Methodological improvements need to be made before cerebral autoregulation testing may become a clinically applicable diagnostic test in the individual. However, the results in this thesis do show that it is possible to measure haemodynamic functioning in a broad range of patients, such as frail elderly and critically ill patients. Using the proposed guideline for transfer function analysis, will make it possible to perform large multi-centre trials with reproducible results in the different centres. It provides a solid standard for comparison between studies performed in different research institutes or at different times, making it possible to pool data and to define clear criteria for 'normal' and 'impaired' cerebral autoregulatory functioning.

Overall, there is a need for signal analysis software programs that describe and analyze systemic and cerebral haemodynamics with 'sense and simplicity', but still in a reliable and valid manner. For further implementation of haemodynamic analysis in both research and clinical practice, the interface between outcome metrics currently used and clinically recognizable results needs to be improved, standardized and simplified as well.

Future research

Outlining the directions in which future research could be most useful, the following research questions should be considered:

Quantification of cerebral autoregulation

Research question 1. How can the proposed guideline for transfer function analysis be further optimized?

The proposed guideline provides a standard which makes it easier to reproduce, compare and implement study results. The guideline reflects what is currently considered best, based on existing literature and expert opinions. However, the evidence in support of some of the proposed settings is still weak and the effect of the usage of different settings for transfer functioning analysis should be further explored. Furthermore, the proposed guideline is only set up for usage in awake and cooperating patients. As cerebral autoregulatory monitoring may also be of benefit in other clinical settings, such as critically ill patients in the intensive care, the guideline should also be adapted to these situations.

Research question 2. Do other methods than transfer function analysis provide better estimates for cerebral autoregulation?

While transfer function analysis is the most applied method for the quantification of cerebral autoregulation in resting state, other methods are available which may provide better estimates for cerebral autoregulation. Different linear methods, i.e. time domain analysis, should be considered, but also the non-linear nature of cerebral autoregulation should be further explored. A number of studies have already shown that the process of cerebral autoregulation contains non-linear dynamics [26], but whether the quantification of the cerebral autoregulatory performance improves when these non-linear dynamics are taken into account has not (yet) been shown.

Research question 3. How can reference values of cerebral haemodynamics in specific populations, such as children, adults, elderly and patients with specific clinical conditions be defined?

Qualifying terms for the interpretation of outcome metrics, for example criteria for “impaired CA”, have never been strictly defined. Reference values for specific populations will make it possible to monitor the quality of cerebral haemodynamics in the individual patient and to investigate the diagnostic usefulness.

Research question 4. How is the cerebral autoregulatory functioning related to other mechanisms, such as the baroreflex functioning?

Cerebral autoregulation is not the only mechanism involved in the control of cerebral perfusion, the exact relationship between cerebral autoregulation and other mechanisms, such as the baroreflex functioning, should be investigated. And, if possible, these mechanisms should be incorporated in the haemodynamic analysis.

Research question 5. How should we define cerebral autoregulation?

While this thesis focused on the measurement techniques for haemodynamic analysis, one should not overlook the fact that, currently, no clear definition on cerebral autoregulation exists. Maybe the concept of cerebral autoregulation, as currently studied, is too limited to cover the whole mechanism at work. Further studies are warranted on what parts of the regulation mechanism should or should not be incorporated within the concept of cerebral autoregulation and a clear definition should be formed.

Clinical application of haemodynamic analysis

Alzheimer's disease

Research question 6. Are the blunted perfusion regulation mechanisms (impaired baroreflex functioning, cerebral autoregulation and cerebrovasomotor reactivity) caused by the pathology of Alzheimer's disease or do they contribute to (accelerate) the pathology of Alzheimer's disease?

In this thesis, we showed that patients with Alzheimer's disease had a blunted vasodilatatory response to hypercapnia (impaired cerebrovasomotor reactivity), an insufficient vasodilatatory and vasoconstrictor responses to decreases and increases in blood pressure (impaired cerebral autoregulation) as well as a lowered baroreflex functioning (Chapter 6 and 7). However, both studies used a cross-sectional design, which makes it impossible to establish whether the blunted perfusion regulation mechanisms are the effect or the cause of neurodegeneration. Knowing more about the causality, may provide useful information toward the understanding, and ultimately, treatment, of Alzheimer's disease.

Frail elderly

Research question 7. Is, in elderly patients with hypotensive syndromes, an impaired cerebral autoregulatory functioning the cause of symptoms such as falls and syncope?

We found that in geriatric patients with hypotensive syndromes, cardiovascular autonomic was not a sufficient cause to explain the occurrence of these hypotensive syndromes (Chapter 9). As symptoms associated with these hypotensive syndromes such as dizziness, lightheadedness, blurred vision and loss of consciousness may point towards insufficient cerebral perfusion, impairment of the cerebral autoregulatory functioning may play a role in these hypotensive syndromes. Investigating the cerebral autoregulatory functioning in patients with hypotensive syndromes may provide important knowledge as cerebral hypoperfusion may place the patient at significant risk to health and may cause injury. Furthermore, as patient complaints can be vague or nonspecific, impaired cerebral autoregulation may be an objective indicator of impaired cerebral perfusion.

Research question 8. Is anti-hypertensive treatment save for elderly patients with hypertension?

Anti-hypertensive treatment can reduce morbidity and mortality risks in hypertensive patients. However, there is an alarming lack of evidence to guide treatment decisions for elderly with hypertension. Therefore, it is difficult for the physician to balance the assumed benefits of treatment against possible risks. This is reflected in the fact that only a small part of hypertensive patients aged >80 years receive adequate treatment. In three hypertensive patients aged between 70 and 80 years, we showed that blood pressure lowering with anti-hypertensive treatment did not result in a reduction of baseline brain blood flow. Validating this finding in a larger group of elderly, will stimulate physicians to treat elderly patients, improving not only the health of the patient but also lowering health expenses as medical interventions which may be needed as hypertension goes untreated can be prevented.

<u>Endotoxemia-induced systemic inflammation</u>

Research question 9. Is the perfusion regulation impaired in septic patients?

The few studies performed during sepsis have shown that cerebral autoregulation is indeed impaired in the great majority of patients. However, in this thesis, we showed that controlled systemic inflammation in healthy volunteers is associated with overall preserved autoregulatory performance at short term and at a later stage. This discrepancy may be caused by the fact that we used the endotoxemia model to study sepsis, but it is also possible that the methods used to quantify cerebral autoregulation (correlation coefficient analysis and transfer function analysis) in the studies using patients with sepsis are not appropriate for this patient group. To rule out that impaired cerebral autoregulatory functioning is one of

the causes of sepsis-associated brain dysfunction, further study on the perfusion regulation in septic patients is needed.

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Chapter 13.

Summary

SUMMARY

Part 1. Quantification of cerebral autoregulation

The term cerebral autoregulation stands for an important concept in cardiovascular regulation, namely that brain circulation is preserved at all cost. Cerebral autoregulation refers to the properties of the brain vascular bed to maintain cerebral perfusion despite changes in blood pressure. Whereas classic studies have assessed cerebral autoregulation during changes in blood pressure that have a gradual onset, dynamic studies quantify the fast modifications in cerebral blood flow in relation to rapid alterations in blood pressure. A large number of methods have been proposed to assess the quality of dynamic cerebral autoregulation. Transfer function analysis is the most frequently used method in the literature to quantify cerebral autoregulation using spontaneous fluctuations in blood pressure and cerebral blood flow velocity. Transfer function analysis is based on analysis of frequency gain, phase and coherence components of oscillations in blood pressure and the resultant degree to which these changes are reflected in cerebral blood flow. One of the advantages of this method is that it only takes a baseline measurement without the need for any pharmacological or physiological manipulation of blood pressure.

In this part of the thesis, the following research question was raised:
How is transfer function analysis applied for the quantification of cerebral autoregulation?

Chapter 2 provides an overview of different ways of execution and implementation of the transfer function in the assessment of cerebral autoregulation. A thorough search of the literature was performed, which yielded 113 studies that had used transfer function to assess cerebral autoregulation for spontaneous oscillations in blood pressure and cerebral blood flow velocity. It was concluded from these studies that there exists a high diversity in settings and criteria used for transfer function analysis. Notable was also the high number of studies with sparse information about the settings of transfer function analysis. The high diversity in the implementation of the transfer function made it difficult to compare the results of the different studies. As a consequence, pooling the data of the different studies for each type of transfer function metric showed an expectedly large between-centre variability. However, the pooled data did reveal multi-centre frequency plot trends for phase and gain that were consistent with the original high pass filter model for dynamic cerebral autoregulation. This implies higher values for phase in the lower frequencies, decreasing with increasing frequency, and lower values for gain in the lower frequencies, increasing with higher

frequency.

To further investigate the between-centre variability, a multi-centre study was performed (**Chapter 3**) to investigate the between-centre variability in transfer function analysis outcome metrics. In this multi-centre study a single, uniform database with healthy patients with blood pressure and transcranial Doppler recordings was analyzed by different research centres, each using their own transfer function settings. A large non-homogeneous variation was found in transfer function outcome metrics between the centres. To evaluate how differences in usual routine settings of the centres might have influenced the outcome of the transfer function analysis, several settings were compared using artificial generated datasets. Results show an urgent need for detailed standardisation of the signal processing methods used for transfer function analysis. Without such standardisation, additional uncertainty is added to any comparison between studies of autoregulation carried out at different centres.

To come to a consensus on the standardisation of transfer function analysis, **Chapter 4** provides preliminary guidelines for the use of transfer function analysis. With these guidelines, we hope to establish more uniformity in the use of transfer function analysis in future literature.

Despite the fact that transfer function analysis is the most widely used method to non-invasively evaluate cerebral autoregulation performance, it is not necessarily the best available method. Transfer function analysis provides an estimate of cerebral autoregulatory performance, but other methods may be equally well or even better able to describe the complex physiological process of cerebral autoregulation. As transfer function analysis is based on the assumption that the relation between blood pressure and cerebral blood flow velocity is linear, while physiologically cerebral autoregulation exhibits nonlinear dynamics, it may be possible that this method oversimplifies the nature of cerebral autoregulation. **Chapter 5,** uses a new non-linear analysis method, convergent cross mapping, to assess these non-linear dynamics of cerebral autoregulation. Convergent cross mapping determines causality between variables by investigating if historical values of the blood pressure can be used to predict the states of the cerebral blood flow velocity. As outcome measure the Pearson correlation between the cerebral blood flow velocity predicted from the blood pressure and the measured cerebral blood flow velocity is used. We have validated this method in 19 healthy adults by comparing normocapnic data with hypercapnic data, which is a model for impaired cerebral autoregulation. The correlation was found to be higher in the hypercapnic state compared to normocapnia, making convergent cross mapping a promising

technique for cerebral autoregulation estimation.

Part 2. Clinical application of haemodynamic analysis

In this part of the thesis, the focus was shifted to haemodynamics in clinical practice. Inspired by the clinical background of the department of geriatric medicine where this thesis research was performed, conditions that are of relevance for an elderly population were investigated. The following research question was investigated:
Is the perfusion regulation impaired in patients with Alzheimer's disease, frail elderly and/ or during systemic inflammation?

Part 2.1. Alzheimer's disease

Alzheimer's disease is a frequent occurring progressive neurodegenerative disorder which places a huge burden on society and individual caregivers. Despite tremendous research efforts aimed at understanding and curing Alzheimer's disease, there are still no simple diagnostic tests for Alzheimer's disease and existing therapies only treat symptoms, but do not actually stop the disease. Recent studies have provided evidence that vascular factors contribute to the neuronal degeneration in Alzheimer's disease and are even considered responsible for the initiation and progression of the neuropathology of Alzheimer's disease. Vascular pathology may impair cerebral autoregulation and lead to cerebrovascular insufficiency, which in turn may contribute to the progression of Alzheimer's disease. Animal studies show strong evidence for impairment of cerebral autoregulation by Alzheimer's disease. However, the exact relationship remains poorly understood. Knowledge about the vascular involvement in the pathogenesis of Alzheimer's disease could lead to the development of adequate techniques for the diagnosis and maybe even effective treatments.

Therefore, in **Chapter 6**, the association of Alzheimer's disease with cerebral autoregulation and cerebral vasomotor reactivity was studied. In this chapter, cerebral autoregulation and cerebral vasomotor reactivity were studied in 12 patients with mild to moderate Alzheimer's disease and 24 controls matched for age and history of hypertension. While transfer function analysis revealed no differences in cerebral autoregulation between the patients with Alzheimer's disease and the controls, the sit-stand manoeuvres showed that Alzheimer patients have insufficient vasodilatatory and vasoconstrictor responses to decreases and increases in blood pressure. Furthermore, a blunted vasodilatatory response to hypercapnia was seen in the patients with Alzheimer's disease, indicating impaired cerebral vasomotor

reactivity.

In **Chapter 7**, we studied whether the baroreflex was less effective in patients with Alzheimer's disease. Baroreflex functioning was assessed in 18 patients with mild to moderate Alzheimer's disease, 11 patients with mild cognitive impairment and 19 age and sex matched healthy controls. We found that the baroreflex functioning was reduced in Alzheimer's disease compared to healthy controls. Baroreflex functioning of patients with mild cognitive impairment was between those with Alzheimer's disease and the healthy controls. Results were confirmed in an independent validation sample of 16 patients with Alzheimer's disease, 18 patients with mild cognitive impairment and 19 age- and sex matched healthy controls. Furthermore, in 18 patients with Alzheimer's disease it was found that treatment with acetylcholinesterase inhibitor increased baroreflex sensitivity with 66%.

The clinical implications of the observed impairments in cerebral autoregulation, cerebral vasomotor reactivity and baroreflex functioning are still unclear and need further research.

Part 2.2. Frail elderly

Age-associated physiological changes in heart rate and blood pressure regulation, comorbid medical conditions such as hypertension and atherosclerosis, and the concurrent use of medication that affect the regulation systems may make elderly highly vulnerable for cerebral hypo- and hyperperfusion. In the three studies, described in **Chapter 8** to **10**, we evaluated haemodynamic changes in elderly patients. Studying healthy elderly (**Chapter 8**), patients with hypertension before and after antihypertensive treatment (**Chapter 9**) and patients with hypotensive syndromes (**Chapter 10**).

Healthy aging

Aging itself leads to a degeneration of the vascular system. Cerebral blood flow decreases, which may influence the vasoregulatory capacity, and vessel stiffness is enhanced. Furthermore, the aging brain shows a compromised microvascular anatomy, which may interact with cerebral brain perfusion and metabolism, contributing to a suboptimal cognitive performance in the elderly. In **Chapter 8** the effect of aging and cognitive load on cerebral haemodynamics is examined using near-infrared spectroscopy, which is a non-invasive tool to measure cortical haemodynamics and metabolism. Fourteen healthy young (>22 years) and 14 healthy older adults (>63 years) performed a verbal n-back working-memory task. It was

found that very low- and low-frequency oscillations of both cerebral haemodynamics and blood pressure were reduced in the older compared to the young adults during task performance. The very low frequency oscillations were also reduced in the group of young persons with increased cognitive load. These results indicate that it is important to take the factors age and cognitive load into consideration for the analysis and interpretation of haemodynamic neuroimaging data. Furthermore, transfer function analysis was used to gain more insight into the relationship between the task-induced oscillations in oxygenated haemoglobin concentration and blood pressure. A clear relationship was found in accordance with the known properties of cerebral autoregulation, which did not change under influence of age and cognitive load.

Hypertension

The prevalence of hypertension in elderly people is increasing. Multiple mechanisms, including stiffening of large arteries, endothelial dysfunction, cardiac remodelling, autonomic dysregulation and renal aspects, contribute to the high prevalence of hypertension in the elderly. Long-standing hypertension may result in structural changes of the cerebral vessels, such as thickening of the vessel walls with narrowing of the lumen and hyalinosis of the media resulting in stiffness. Several studies have demonstrated that antihypertensive drug therapy reduces the development of new coronary events, stroke and congestive heart failure in elderly persons. However, at short term antihypertensive drug therapy may also have disadvantages. Indications exist that hypertension impairs the mechanism of cerebral autoregulation itself plus it could attenuate orthostatic hypotension. **Chapter 9** represents a case report of three hypertensive patients. Haemodynamic changes after antihypertensive treatment in these patients were studied. It was found that cerebral perfusion (regulation) is preserved in hypertensive elderly before and after antihypertensive treatment.

Hypotensive syndromes

Three other disorders of the blood pressure regulation which are highly prevalent in the elderly are orthostatic hypotension, postprandial hypotension and carotid sinus hypersensitivity. These hypotensive syndromes are characterized by a fall in blood pressure when a person assumes a standing position, a drastic decline in blood pressure after eating a meal and an exaggerated response to carotid sinus baroreceptor stimulation, respectively. Though highly prevalent, the underlying pathophysiology is not clearly understood. In **Chapter 10**, several non-invasive cardiovascular function indices to assess heart rate

variability, blood pressure variability and baroreflex functioning were studied in 203 patients, who visited the falls and syncope outpatient clinic of the Radboud university medical centre because of falls, dizziness and/or syncope. No differences were found in cardiovascular function indices between patients with and without hypotensive syndromes. Symptoms, such as falls, dizziness or syncope, could also not be related to changes in cardiovascular functioning. These findings argue against a single pathological factor, such as autonomic dysfunctioning, causing these hypotensive disorders. It is more likely that these syndromes, especially in the elderly, are multifactorial in genesis.

Part 2.3. Endotoxemia-induced systemic inflammation

Sepsis is an adverse systemic response to infection that includes fever, rapid heartbeat and respiration, low and unstable blood pressure and organ dysfunction associated with compromised circulation. Acute brain dysfunction is often one of the first symptoms of sepsis and may herald an unfavourable neurocognitive outcome. An important precipitating factor for the neurocognitive decline may be a reduced cerebral blood flow due to a reduced capacity of cerebral autoregulation to compensate for the reduction in and instability of cerebral perfusion pressure (i.e. blood pressure).

In **Chapter 11,** cerebral autoregulation was studied during endotoxemia-induced systemic inflammation in 16 healthy young men. Blood pressure and cerebral blood flow velocity were assessed 2 hours before and 2, 4, 6 and 8 hours after infusion of a low dose of purified *E. coli* lipopolysaccharide. At each time point, cerebral autoregulatory performance was quantified in the time domain and frequency domain using correlation coefficient analysis and transfer function analysis, respectively. Despite the marked systemic inflammatory response after LPS infusion similar to that of the very early stages of sepsis, the serial measurements over time revealed no deterioration of the cerebral autoregulation.

Chapter 14.

Nederlandse samenvatting (Summary in Dutch)

Dankwoord (Acknowledgments)

Curriculum Vitae

List of publications

NEDERLANDSE SAMENVATTING (SUMMARY IN DUTCH)

De hersenen, die twee procent van het lichaamsgewicht uitmaken, verbruiken twintig procent van alle zuurstof die in een lichaam wordt opgenomen. De zuurstofvoorziening van de hersenen is afhankelijk van de hoeveelheid zuurstof in het bloed en de hoeveelheid bloed die naar de hersenen stroomt. Onderbreking van de bloedtoevoer van enkele seconden kan al leiden tot een verlies van bewustzijn en uiteindelijk zelfs tot hersenschade. De hersenen zijn dus sterk afhankelijk van een goede en zo stabiel mogelijke toevoer van bloed met zuurstof en glucose. Omdat de bloeddruk ook onder gezonde omstandigheden varieert (bijvoorbeeld doordat we schrikken of ineens opstaan), kent het lichaam verscheidene mechanismen om de bloedstroom naar de hersenen op peil te houden. De belangrijkste regulatie mechanismen van het lichaam om de doorbloeding van de hersenen te reguleren zijn de controle van de bloeddruk via de baroreflex en die van de hersendoorbloeding zelf via de cerebrale autoregulatie (BOX 1). De baroreflex zorgt ervoor dat het hart sneller gaat slaan en vaten vernauwen wanneer de bloeddruk daalt en visa versa het hartritme vertraagd en vaten verwijden wanneer de bloeddruk stijgt. Het is een feedbacksysteem dat de bloeddruk op peil houdt. Naast het baroreflex zorgt de cerebrale autoregulatie voor een demping van overdracht van de schommelingen in bloeddruk op de daadwerkelijke doorbloeding van de hersenen. De cerebrale autoregulatie reguleert de locale bloedtoevoer door aanpassingen van de diameter van de vaten in de hersenen.

Wanneer deze regulatie mechanismen falen wordt de doorbloeding van de hersenen sterk afhankelijk van de bloeddruk. Hierdoor daalt de hersendoorbloeding bij bijvoorbeeld een daling van de bloeddruk (vb. bij het opstaan) en dit is niet zonder gevaar, maar kan onder andere leiden tot het ontstaan van hersenschade. Het goed functioneren van de regelmechanismen is dus van groot belang. De literatuur laat echter zien dat verschillende aandoeningen, zoals beroerte, dementie en hoofdtrauma, deze belangrijke regelsystemen kunnen beïnvloeden. Dit kan zorgen voor een verhoogd risico op hersenschade in patiënten met deze aandoeningen.

In de kliniek kan het meten van het functioneren van de baroreflex en cerebrale autoregulatie dus een grote meerwaarde hebben voor de behandeling van patiënten met bijvoorbeeld hersenschade, meningitis of beroerte. Maar het kan ook van belang zijn voor de vroege detectie van neurodegeneratieve ziekten zoals dementie. Vooruitgang in meettechnieken en rekenmethoden hebben het mogelijk gemaakt om stabiele en dynamische veranderingen in hartritme, bloeddruk en hersendoorbloeding te bestuderen. Helaas is er momenteel nog

BOX 1.

Baroreflex

De baroreflex is een belangrijk regelmechanisme, welke zorgt voor instandhouding van de adequate bloeddruk in het lichaam. Deze reflex werkt zeer snel en komt in actie bij plotselinge drukveranderingen, waarbij door middel van aanpassingen van de vaatdiameter en het hartritme de bloeddruk gecorrigeerd. De baroreflex werkt door middel van baroreceptoren, rekgevoelige vezels in bijvoorbeeld de wand van de aorta. Wanneer iemand plotseling opstaat vanuit liggende houding, dan wordt het bloed door de zwaartekracht in de richting van de benen getrokken. De daling van de bloeddruk zorgt dat de baroreceptoren signalen van een verminderde uitrekking afgeven signalen af welke via de hersenen uiteindelijke resulteren in een verwijding van de bloedvaten en een versnelling van het hartritme.

Cerebrale autoregulatie

Cerebrale autoregulatie is een mechanisme waarbij de hersenen de doorbloeding zo stabiel mogelijk houden door dynamische aanpassing van de vaatweerstand aan wisselingen in de bloeddruk. Daalt de bloeddruk dan worden de hersenvaten verwijd, waardoor deze meer bloed kunnen verwerken, en bij een stijging van de bloeddruk vindt vernauwing van de vaten plaats. Op deze wijze kunnen geleidelijke veranderingen in bloeddruk tussen de 50 en 150 mmHg opgevangen worden. Buiten deze range worden bloeddruk schommelingen passief doorgegeven aan de hersendoorbloeding, doordat de hersenvaten niet verder in staat zijn te vernauwen of te verwijden. De aanpassingen van de vaten op geleidelijke veranderingen wordt 'statische autoregulatie' genoemd en wordt vaak onderzocht door grote bloeddrukschommelingen te induceren, bijvoorbeeld door toediening van geneesmiddelen, en de mate van verandering in hersendoorbloeding te meten. Naast de statische autoregulatie zorgt de zogenaamde dynamische autoregulatie voor aanpassingen van de vaten op plotselinge veranderingen (binnen secondes) in de bloeddruk. Een goud standaard voor het meten van de dynamische autoregulatie bestaat momenteel nog niet.

geen goud standaard techniek om de kwaliteit van de cerebrale autoregulatie te meten.

Dit proefschrift is opgedeeld in twee delen, waarvan het eerste deel gewijd is aan de kwantificatie van cerebrale autoregulatie. Hierbij ligt de focus op de meest gebruikte niet-invasieve methode op dit moment, genaamd overdrachtsfunctie analyse. De toepassing van deze methode is in kaart gebracht door middel van een review van de literatuur en een multi-center studie. Om tot standaardisatie van de techniek te komen, hebben we een voorstel gedaan voor richtlijnen omtrent de toepassing van de overdrachtsfunctie analyse voor het meten van cerebrale autoregulatie. Het tweede deel bevat studies naar het functioneren van de doorbloedingsregulatie in verschillende patiënten populaties. Geïnspireerd door de klinische achtergrond van de afdeling waar de studies beschreven in dit proefschrift werden uitgevoerd, werden aandoeningen die van belang zijn voor de oudere, waaronder de ziekte van Alzheimer, hypertensie en orthostatische hypotensie, onderzocht.

Dit hoofdstuk bevat een samenvatting van de bevindingen in dit proefschrift.

Deel 1. Kwantificatie van cerebrale autoregulatie

In dit deel van het proefschrift staat de volgende onderzoeksvraag centraal: Hoe wordt overdrachtsfunctie analyse toegepast voor de kwantificering van cerebrale autoregulatie?

Ondanks de grote meerwaarde welke het meten van de cerebrale autoregulatie in de klinische praktijk kan hebben, bestaat er op dit moment nog geen goud standaard methode hiervoor. Overdrachtsfunctie analyse is momenteel de meest gebruikte niet-invasieve methode om cerebrale autoregulatie te kwantificeren. Om de overdrachtsfunctie analyse te kunnen toepassen is een simultane meting van de bloeddruk en hersendoorbloeding nodig. Zowel de bloeddruk als de hersendoorbloeding kunnen niet-invasief worden gemeten (zie Figuur 1A). Zowel de bloeddruk als de hersendoorbloeding zijn opgebouwd uit schommelingen met verschillende frequenties. Met behulp van de overdrachtsfunctie analyse kan in kaart worden gebracht hoe (vb. versterking en vertraging) verschillende schommelingen in de bloeddruk worden doorgegeven aan de hersenendoorbloeding. Het is bekend dat de cerebrale autoregulatie werkt als een hoog-doorlaatfilter, dit betekent dat langzame schommelingen in de bloeddruk (lage frequenties) worden gedempt en snelle schommelingen in de bloeddruk (hoge frequenties) passief worden doorgegeven aan de hersendoorbloeding (Figuur 1B). Wanneer de overdrachtsfunctie niet het kenmerkende hoog-doorlaatfilter weergeeft is er sprake van een verstoring van de cerebrale autoregulatie. Een

van de voordelen van deze methode is dat er geen farmacologische of fysiologische manipulatie van de bloeddruk nodig is, maar dat een rustmeting voldoende is.

Om te onderzoeken of de overdrachtsfunctie analyse gebruikt zou kunnen worden als goud standaard voor de kwantificering van cerebrale autoregulatie, hebben we een review van de literatuur (**Hoofdstuk 2**) en een multi-center studie (**Hoofdstuk 3**) uitgevoerd.

Met het review hebben we een overzicht gemaakt van hoe de overdrachtsfunctie voor de kwantificatie van de cerebrale autoregulatie momenteel wordt toegepast in de literatuur. Van 113 studies werd de wijze van uitvoering van de overdrachtsfunctie in kaart gebracht. Het review laat een grote diversiteit aan wijze van uitvoering van de overdrachtsfunctie analyse en criteria voor de interpretatie van de uitkomsten zien tussen de studies. Daarnaast was opvallend dat een groot deel van de studies niet hun gehele procedure beschreven hadden in het artikel. Ondanks dat de studies hun resultaten dus allen presenteerden onder de noemer 'verkregen met overdrachtsfunctie analyse' verschilde de wijze van overdrachtsfunctie dus onverklaarbaar veel. Omdat het effect van de wijze waarop de overdrachtsfunctie wordt uitgevoerd op de uiteindelijke uitkomsten voor cerebrale autoregulatie onbekend is, maakt deze grote diversiteit in de uitvoering van de overdrachtsfunctie het moeilijk om de resultaten van de verschillende onderzoeken te vergelijken. Het doel van de multi-center studie (beschreven in **Hoofdstuk 3**) was te onderzoeken of de wijze van uitvoering van de overdrachtsfunctie analyse invloed heeft op de uitkomst voor cerebrale autoregulatie. Voor deze studie werden 15 centra gevraagd dezelfde 80 datasets te analyseren met hun eigen instellingen voor de overdrachtsfunctie analyse. Grote verschillen in uitkomsten voor de cerebrale autoregulatie van de 80 datasets tussen de centra werden waargenomen. Hierdoor werd duidelijk dat uitvoering van de overdrachtsfunctie op de ene wijze de patiënt zou diagnosticeren als hebbende een goede cerebrale autoregulatie, terwijl een andere wijze op een verstoorde cerebrale autoregulatie uit zou komen.

Om verschillende studies met elkaar te kunnen vergelijken is standaardisatie van de uitvoering van de overdrachtsfunctie analyse dus van belang. In **Hoofdstuk 4** wordt een eerste voorzet gegeven voor een richtlijn omtrent het gebruik van de overdrachtsfunctie analyse. Met deze uniforme methode kan internationaal vervolgonderzoek worden gedaan met de grote winst dat uitkomsten nu te vergelijken en combineren zijn. Daarnaast maakt standaardisatie van de methode het mogelijk om de sensitiviteit, specificiteit en robuustheid van de overdrachtsfunctie te vergelijken met andere beschikbare methoden.

Ondanks dat de overdrachtsfunctie analyse de meest gebruikte niet-invasieve methode is om cerebrale autoregulatie te kwantificeren is op dit moment nog onbekend of het ook de beste methode is. Het feit dat de overdrachtsfunctie analyse gebaseerd is op de veronderstelling dat er een lineaire relatie is tussen bloeddruk en hersendoorbloeding, maakt dat verscheidene onderzoekers de accuraatheid van de methode in twijfel trekken en voorkeur geven aan een niet-lineaire methode.

In **Hoofdstuk 5** wordt een nieuwe niet-lineaire analyse methode, genaamd convergend cross mapping, voor de cerebrale autoregulatie beschreven. Convergend cross mapping bepaalt de causaliteit tussen schommelingen in hersendoorbloeding en bloeddruk door te onderzoeken of de waarden van de bloeddruk in het verleden kunnen worden gebruikt om de hersendoorbloeding te voorspellen.

Deel 2. Toepassing van haemodynamische analyses in de klinische praktijk

In dit deel van het proefschrift ligt de focus op haemodynamica in de klinische praktijk. De volgende onderzoeksvraag werd onderzocht: Is de regulering van de hersenperfusie aangetast in patiënten met de ziekte van Alzheimer, kwetsbare ouderen en / of tijdens een systemische ontsteking?

Deel 2.1. De ziekte van Alzheimer

De ziekte van Alzheimer is een frequent voorkomende progressieve neurodegeneratieve ziekte. Ondanks de grote hoeveelheid onderzoek die gedaan wordt op het gebied van Alzheimer, zijn er nog geen eenvoudige diagnostische testen beschikbaar voor de ziekte van Alzheimer. Daarnaast behandelen bestaande therapieën alleen de symptomen, maar stoppen niet daadwerkelijk de ziekte. Recent hebben studies aangetoond dat ook vasculaire factoren bijdragen aan de neuronale degeneratie bij de ziekte van Alzheimer en mogelijk zelfs verantwoordelijk zijn voor de initiatie en progressie van de neuropathologie. Vasculaire pathologie kan de cerebrale autoregulatie beïnvloeden en leiden tot cerebrovasculaire insufficiëntie, welke weer kan bijdragen aan de progressie van de ziekte van Alzheimer. Kennis over de vasculaire betrokkenheid in het ontstaan van de ziekte van Alzheimer kan helpen bij de ontwikkeling van betere diagnostische technieken en mogelijk zelfs effectievere behandelingen. In de studies beschreven in **Hoofdstuk 6** en **7** hebben we onderzocht of de cerebrale autoregulatie, cerebrale vasomotore reactiviteit en/of het baroreflex functioneren verminderd zijn in patiënten met de ziekte van Alzheimer.

In **Hoofdstuk 6** is de cerebrale autoregulatie en cerebrale vasomotore reactiviteit onderzocht in 12 patiënten met milde tot matige Alzheimer en 24 controles welke gematched waren voor leeftijd en op voorgeschiedenis van hypertensie. Met behulp van zit-sta manoeuvres toonden we aan dat Alzheimer patiënten grotere schommelingen in hersendoorbloeding laten zien in reactie op schommelingen in de bloeddruk dan de controles. Dit wijst op onvoldoende vasodilatatoire en vasoconstrictoire en is dus een teken van een verstoorde cerebrale autoregulatie. De vasomotore reactiviteit werd gemeten door de proefpersonen een gasmengsel met een verhoogde CO_2 concentratie te laten inademen voor ongeveer 30 sec. De verhoogd CO_2 concentratie zorgt ervoor dat de hersenvaten verwijden en de hersendoorbloeding stijgt. In de patiënten met de ziekte van Alzheimer werd een verminderde vasodilatatoire reactie waargenomen, wat aangeeft dat ook de vasomotrische reactiviteit verminderd is.

Hoofdstuk 7 laat zien dat, naast de cerebrale autoregulatie en de cerebrale vasomotore reactiviteit, ook het baroreflex minder effectief werkt bij patiënten met de ziekte van Alzheimer. Het baroreflex functioneren is onderzocht in twee onafhankelijke centra waarbij patiënten met milde tot matige ziekte van Alzheimer, patiënten met milde cognitieve stoornissen en gezonde controles zijn geïncludeerd. In beide centra werd een verminderd functioneren van het baroreflex gevonden bij patiënten met milde cognitieve stoornissen ten opzichte van de gezonde controles. Daarnaast werd ook een verminderde baroreflex functie gevonden in de groep patiënten met de ziekte van Alzheimer ten opzichte van de patiënten met milde cognitieve stoornissen. Dit suggereert dat naast de cognitie ook de regulering van de bloeddruk al in een vroeg stadium van de ziekte van Alzheimer verstoord raakt. Naast dat de verstoring van het baroreflex functioneren meer verteld over het verloop van de ziekte van Alzheimer, kan de baroreflex functie mogelijk ook worden gebruikt als diagnostisch middel voor het opsporen van de ziekte van Alzheimer in een vroeg stadium.

Omdat de waargenomen stoornissen in hersenperfusie regulering subtiel zijn, leiden ze waarschijnlijk niet direct tot ernstige hypoperfusie en daarmee niet tot (ernstige) cerebrale ischemie. Toch is het niet ondenkbaar dat chronische milde hypoperfusie bijdraagt aan de atrofie van de hersenen bij de ziekte van Alzheimer en is het van belang om de klinische implicaties van de waargenomen stoornissen verder te onderzoeken.

<u>Deel 2.2. Kwetsbare ouderen</u>

Het aantal ouderen neemt toe. In Nederland zullen er in 2040 naar schatting 4,6 miljoen 65-

plussers zijn. Dat ouderen een hoger risico hebben op cerebrale hypo- en hyperperfusie is aan verschillende oorzaken toe te schrijven, zoals leeftijdsgeassocieerde fysiologische veranderingen in de hartslag en bloeddruk regulatie, comorbide medische aandoeningen zoals hypertensie en atherosclerose en het gebruik van medicatie. In dit deel van het proefschrift hebben we de haemodynamiek bestudeerd van gezonde ouderen (**Hoofdstuk 8**), oudere patiënten met hypertensie voor en na de behandeling met antihypertensiva (**Hoofdstuk 9**) en van oudere patiënten met een hypotensieve syndromen (**Hoofdstuk 10**).

Gezond ouderen

Tijdens het ouder worden gaat de kwaliteit van het vasculaire systeem langzaam achteruit. De hersendoorbloeding neemt af, hetgeen de vasoregulatoire capaciteit kan beïnvloeden, en de vaatstijfheid neemt toe. Bovendien tonen de ouder wordende hersenen een verminderde microvasculaire anatomie. Samen zorgen deze factoren ervoor dat de cognitieve prestaties van ouderen achteruitgaan. In **Hoofdstuk 8** hebben we het effect van veroudering en cognitieve belasting op de cerebrale haemodynamiek onderzocht. Hiervoor is gebruik gemaakt van *near-infrared spectroscopy*. Door middel van deze techniek kan niet-invasief een meting van de cerebrale oxygenatie worden verricht op basis van het verschil in lichtabsorptie tussen geoxygeneerd en gedeoxygeneerd hemoglobine in het brein.

Veertien gezonde jonge (> 22 jaar) en 14 gezonde oudere volwassenen (> 63 jaar) deden mee aan het onderzoek. Een verminderde hoeveelheid langzame schommelingen in zowel de bloeddruk als de cerebrale oxygenatie werd waargenomen in de groep gezonde ouderen. Wanneer de jongeren verhoogd cognitief belast werden, door middel van een verbale werkgeheugentaak, werd ook bij hen een vermindering van de hoeveelheid langzame schommelingen waargenomen. Dit suggereert dat ouderen al eerder een stadium van hogere cognitief belasting bereiken. De cerebrale autoregulatie bleek niet aangetast te worden door de veroudering zelf.

Hypertensie

Hypertensie is een aandoening waarbij er sprake is van een te hoge bloeddruk. Door leeftijdsgeassocieerde fysiologische veranderingen, zoals een verhoogde vaatstijfheid van grote slagaders, endotheliale dysfunctie en autonome ontregeling kampt een groot gedeelte van de ouderen met hypertensie. Langdurige hypertensie is echter niet zonder nadelige gevolgen. Het kan leiden tot structurele veranderingen van de hersenvaten. Verschillende studies hebben aangetoond dat medicatie om de bloeddruk te verlagen het risico op bijvoorbeeld een beroerte en hartfalen vermindert. Echter, is er de kans dat op korte termijn

bloeddrukverlagende medicatie ook nadelige effecten hebben. Aanwijzingen bestaan dat hypertensie de functie van de cerebrale autoregulatie vermindert en orthostatische hypotensie (een sterke bloeddruk daling bij het opstaan) versterkt. Het verlagen van de bloeddruk met medicatie kan daarom leiden tot hypoperfusie van de hersenen. Om te bekijken of dit het geval is, hebben we in een case studie van drie oudere patiënten met hypertensie (**Hoofdstuk 9**) onderzocht of er haemodynamische veranderingen gepaard gaan met bloeddrukverlagende medicatie in oudere patiënten met hypertensie. Uit deze case studie kwamen geen aanwijzingen voor een vermindering van de hersendoorbloeding na het gebruik van bloeddrukverlagende medicatie naar voren. Dit suggereert dat het veilig is om bloeddrukverlagende medicatie voor te schrijven in deze patiënten groep.

Hypotensieve syndromen

Drie veel voorkomende andere aandoeningen in ouderen die te maken hebben met de bloeddruk regulering zijn orthostatische hypotensie, postprandiale hypotensie en sinus carotis hypersensitiviteit. Deze zogenaamde hypotensieve syndromen worden gekenmerkt door een flinke daling van de bloeddruk. Bij orthostatische hypotensie vindt deze bloeddrukdaling plaats na het gaan staan, bij postprandiale hypotensie na het eten van een maaltijd en bij sinus carotis hypersensitiviteit ontstaat de bloeddrukdaling als gevolg van een overdreven reactie op de stimulatie van sinus carotis baroreceptor. Ondanks dat de syndromen veel voorkomen, is de onderliggende pathofysiologie nog onduidelijk. In de studie beschreven in **Hoofdstuk 10** hebben we daarom onderzocht of er verstoringen zijn in de cardiovasculaire regelsystemen die gepaard gaan met deze syndromen. Hiervoor hebben we gekeken naar de hartslag variabiliteit, bloeddruk variabiliteit en het functioneren van het baroreflex in 203 patiënten, wie vanwege klachten van vallen, duizeligheid en/of syncope onze val en syncope polikliniek van het Radboud universitair medisch centrum bezochten. Geen verschillen in de reguleringsmaten werden waargenomen tussen patiënten met en zonder hypotensieve syndromen. Ook symptomen, zoals vallen, duizeligheid en syncope, konden niet worden gerelateerd aan de werking cardiovasculaire systemen. Deze resultaten geven aan dat het onwaarschijnlijk is dat er één pathologische factor is, zoals autonome disfunctie, welke de aandoening deze aandoeningen zou veroorzaken. Het is waarschijnlijker dat vele verschillende factoren samen ten grondslag liggen aan deze syndromen en dat daarom een verschillen in de enkele maten gevonden konden worden.

Deel 2.3. Endotoxemia geïnduceerde systemische ontsteking

Sepsis (in de volksmond ook wel ‘bloedvergiftiging’) is een ontstekingsreactie van het gehele

lichaam, als reactie op een infectie. Het is een ernstig verlopend ziektebeeld welke gekenmerkt wordt door een snelle hartslag en ademhaling, lage en instabiele bloeddruk en orgaan disfunctie. Sepsis leid in veel gevallen tot de dood. Eén van de eerste symptomen van sepsis is acuut hersenfalen, wat vaak een ongunstige neurocognitieve toestand tot gevolgd heeft. Een belangrijke factor in het ontstaan van een verminderde neurocognitieve toestand kan een verlaagde hersenperfusie zijn. Deze verlaagde hersenperfusie kan een gevolg van een verminderd functioneren van de cerebrale autoregulatie. Om te onderzoeken hoe de cerebrale autoregulatie functioneert tijdens sepsis, hebben we de cerebrale autoregulatie onderzocht tijdens een endotoxine-geïnduceerde systemische ontsteking in 16 gezonde jonge mannen (**Hoofdstuk 11**).

De bloeddruk en cerebrale bloeddoorstromingssnelheid werden 2 uur voor en 2 , 4 , 6 en 8 uur na infusie van een lage dosis van gezuiverde *E. coli* lipopolysaccharide gemeten. Op elk tijdstip werd het functioneren van de cerebrale autoregulatie gekwantificeerd in het tijdsdomein en het frequentiedomein middels correlatie coëfficiënt analyse en overdrachtsfunctie analyse. Ondanks de sterke systemische ontstekingsreactie (vergelijkbaar met de symptomen die voor komen in de zeer vroege stadia van sepsis) die werd waargenomen in de proefpersonen vonden we geen verslechtering van de cerebrale autoregulatie.

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DANKWOORD (ACKNOWLEDGMENTS)

Piglet:
'How do you spell love?'
Pooh:
'You don't spell it, you feel it.'

Het is eindelijk zover...
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Lieve Eva, sis, ook al heb je het misschien niet altijd gemakkelijk met zo'n betweterige oudere zus, samen hebben we (bijna) altijd lol. Forever sisters!

Lieve Daniël, je moest het allemaal aanhoren en meemaken; mijn gemopper als het niet wilde vlotten, de statistiekperikelen waar ik je mee opscheepte, de zenuwen als ik op een buitenland bezoek moest, alle keren dat ik weg moest voor een cursus of congres (in plaats van gezellig bij jou te zijn), alle presentaties waar je naar mocht luisteren. Dank je wel voor al je steun en liefde. Het is heerlijk samen met jou!

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CURRICULUM VITAE

Aisha Meel-van den Abeelen (19 april 1987) groeide op in Hengelo (Overijssel). Na het behalen van het Gymnasium diploma op de Grundel te Hengelo, startte zij in 2005 met de studie Technische Geneeskunde aan de Universiteit Twente te Enschede.

Na haar eerste masterstage op de afdeling klinische neurofysiologie van het Radboudumc, volgden vele stages in het Radboudumc (KNO, Geriatrie en NICU). In 2011 behaalde zij haar universitaire diploma, waarna ze verder ging als OIO op de afdeling geriatrie in het Radboudumc. Haar promotieonderzoek naar de kwantificatie en klinische toepassing van de cerebrale autoregulatie werd kort onderbroken voor de komst van een prachtige dochter Livia. Daarna heeft ze haar Ph.D. traject weer opgepakt. Sinds juni 2014 werkt ze als post-doctoraal onderzoeker aan een onderzoek naar de toepassing van 3D-ultrasound voor borstkankerdiagnostiek.

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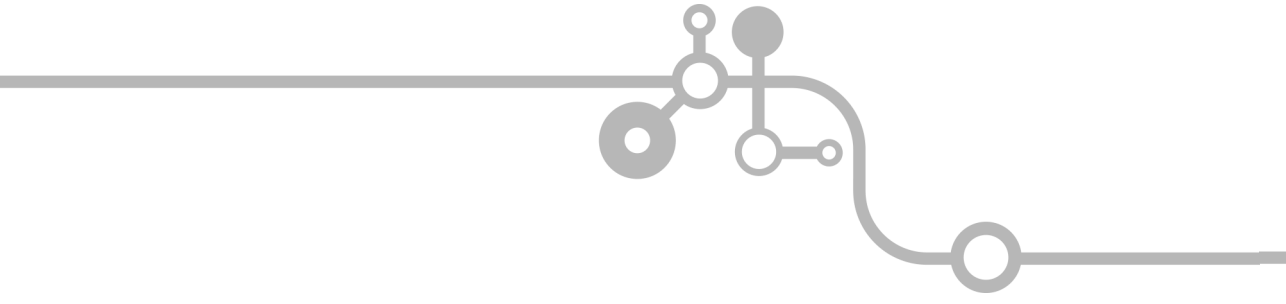

‘Promise me you’ll always remember:
you’re braver than you believe,
and stronger than you seem,
and smarter than you think.’

-Pooh-