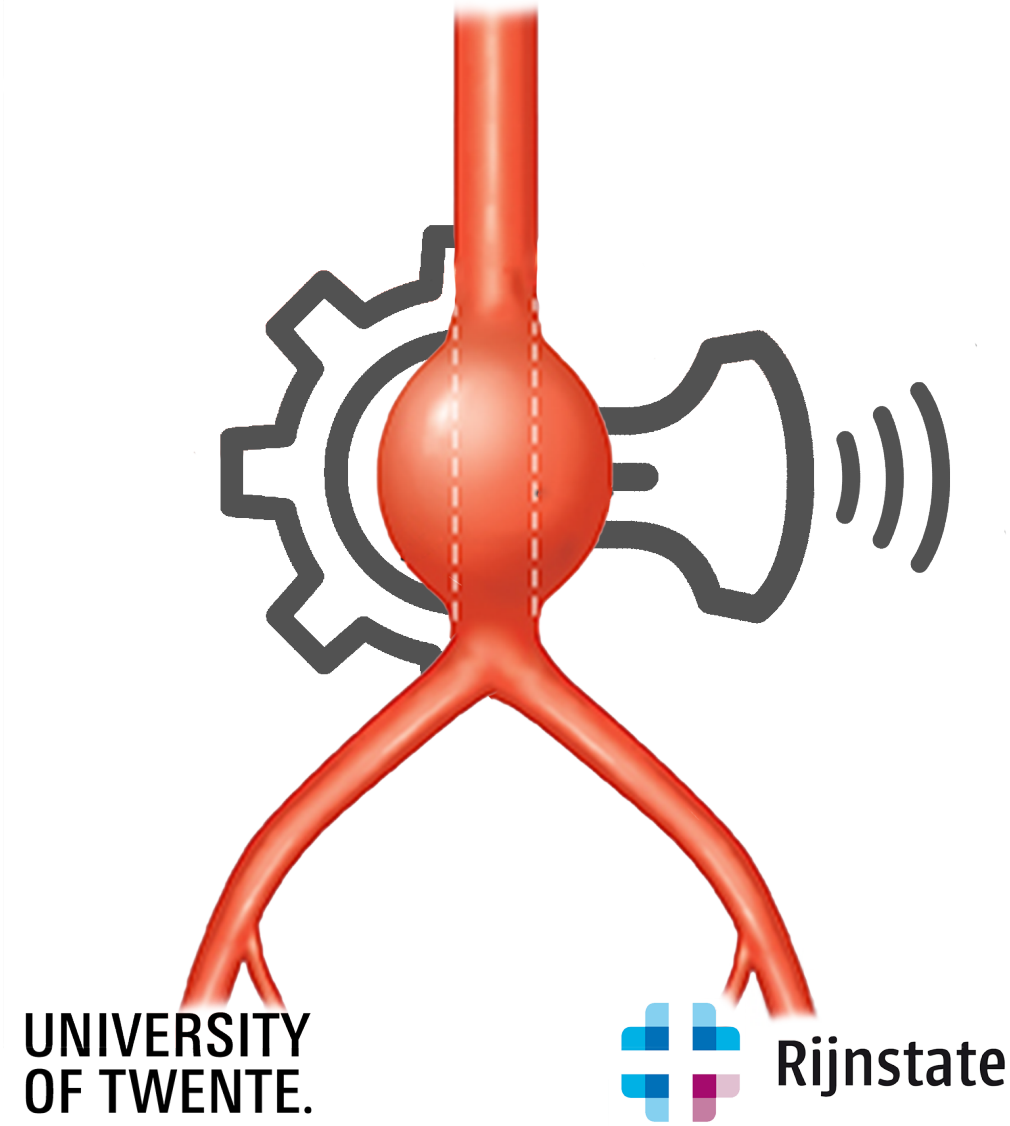
Measuring vasomotor response in abdominal aortic aneurysm patients; a cool innovation toward cardiovascular event prediction

A.S. Jansen

Master thesis: Technical Medicine, Medical Sensing and Stimulation
University of Twente, Department of Vascular Surgery at Rijnstate Hospital Arnhem

April 9, 2020



UNIVERSITY OF TWENTE.

Rijnstate

Graduation committee:

Prof. dr. M.M.P.J. REIJNEN	*Chairman, Medical supervisor*
Dr. E. GROOT JEBBINK	*Technical supervisor*
Drs. P.A. VAN KATWIJK	*Process supervisor*
J.J.M. VERMEULEN, MSc	*Daily supervisor*
Dr. ir. B.E. WESTERHOF	*External supervisor*

Abstract

Objective: To develop an objective method to examine the vasomotor response and investigate this vasomotor response in the common carotid artery (CCA) and the abdominal aorta in both healthy participants and abdominal aortic aneurysm (AAA) patients. Future studies will use this information to develop a method to predict cardiovascular events in AAA patients.

Method: The vasomotor reactivity test (VRT) was performed on 20 healthy young subjects and 11 AAA patients. The VRT consisted of a ultrasound measurement during baseline (30 sec) and the exposure of one hand into ice water (3 min). The ultrasound measurements of both arteries were performed simultaneously. The vasomotor response was defined by mean baseline diameter, area under the curve (AUC), max diameter change, max peak, time to peak, slope, duration of response, AUC of response, effective diameter change and impact factor. The parameters were compared for both arteries and both groups.

Results: The maximum diameter change for the abdominal aorta is 4.9% [2.5,8.2] in the healthy young group, and -2.3% [-3.7,0.7] in the AAA group. The maximum diameter change for the CCA is 3.5% [-1.8,5.7] in the healthy group versus 1.4% [-3.0,2.2] in the AAA group. More parameters were significantly different for the abdominal aorta than the CCA when comparing both groups. The healthy group showed more vasodilation in response to the VRT than the AAA group for both arteries.

Conclusion: The parameters can objectively examine the vasomotor response. The vasomotor response of the abdominal aorta and CCA is altered in the presence of an AAA, which is promising for the development of a method to predict cardiovascular events.

Contents

Abstract **2**

Contents **3**

Glossary **4**

Introduction **4**

1 Background **9**
1.1 Abdominal aortic aneurysm . . . 9
1.2 Vasomotor reactivity test . . . 11

2 Technical challenges and definitions **15**
2.1 Ultrasound specifications during the VRT . . . 15
2.2 Analyzing the vasomotor response during the VRT: a refined method . . . 15

3 RESPONSE study: Relation betwEen abdominal aorta and carotid artery responses to SymPathetic stimulatiON uSing duplEx ultrasound **19**
3.1 Introduction . . . 19
3.2 Method . . . 20
3.3 Results . . . 22
3.4 Discussion . . . 27
3.5 Conclusion . . . 31

4 Spectral analysis **33**
4.1 Introduction . . . 33
4.2 Method . . . 34
4.3 Results . . . 34
4.4 Discussion . . . 35
4.5 Conclusion . . . 35

5 Abdominal probe test **37**
5.1 Abdominal probe test: without VRT . . . 37
5.2 Abdominal probe test: during VRT . . . 40

6 Future perspective **43**

7 Case report: Embolization of the false lumen of an infrarenal post-dissection aneurysm using IMPEDE-FX Embolization Plugs **45**
7.1 Abstract . . . 45
7.2 Introduction . . . 45
7.3 Case report . . . 46
7.4 Discussion . . . 47

Bibliography **49**

8 Appendix **57**
8.1 Rose-questionnaire 57

Glossary

3D Three-dimensional. 43

AAA Abdominal aortic aneurysm. 2, 7–13, 15, 19–22, 24–31, 43, 44

AUC Area under the curve. 2, 15–17, 21, 22, 24–29, 33

AVI Audio video interleave. 21, 30, 34, 38

BP Blood pressure. 20–22, 28

CA Carotid artery. 7, 11, 12, 19, 28, 29

CAR Carotid artery reactivity. 11–13, 19

CCA Common carotid artery. 2, 8, 13, 15, 19–22, 24–31, 33, 43, 44

CPT Cold pressor test. 7, 11–13, 15, 16, 19–21, 28, 34, 40–42

CV Cardiovascular. 19, 20, 31

CVD Cardiovascular disease. 19

EVAR Endovascular aneurysm repair. 10, 31, 44

HR Heart rate. 33–35, 43

IQR Interquartile range. 21, 22

MAD Median absolute deviation. 15, 21, 34

OSR Open surgical repair. 10

PAD Peripheral arterial disease. 12, 20, 30, 43

PCC Pearson's correlation coefficient. 21, 22, 24, 25

PSD Power spectral density. 33

rAAA Ruptured abdominal aortic aneurysm. 7, 10

ROI Region-of-interest. 21, 30

RR Respiratory rate. 33–35, 43

SAM Sympatho-adrenomedullary. 11

SMCs Smooth muscle cells. 9, 11, 28, 29

US Ultrasound. 7, 10–13, 15, 19, 20, 22, 30, 34, 35, 37, 38, 40, 41, 43

VRT Vasomotor reactivity test. 2, 3, 7, 8, 11–13, 15, 16, 19–22, 27–31, 34, 35, 37–44

Introduction

The incidence of a pathological widening of the abdominal aorta, an abdominal aortic aneurysm (AAA) is about 1.7% for women and 5% for men [1]. The prevalence and incidence rate are decreasing over the last 20 years, partially because of an increased smoking cessation [2–4]. AAA prevalence increases with age, with a prevalence rate of 351 per 100.000 at the age of 55-59 years and 726 per 100.000 at the age of 60-64 years [2].

When an AAA diameter is greater than 5.5 cm for men and 5.0 cm for women or grows more than 1 cm per year, there is an increased risk of rupture and the risks of surgery outweigh the risk of rupture [1, 5]. Therefore, AAAs must be detected in an early stage, return for regular follow-up and if necessary treated. Most AAAs are asymptomatic and are therefore only detected as an incidental finding when using imaging techniques for other indications, e.g. CT, MRI or ultrasound [1]. Although some AAAs may manifest with abdominal or back pain, in many cases the first symptom is rupture (rAAA). The mortality rate of an rAAA is >80%, and about 32% percent of the patients with rAAA died before reaching the hospital [6]. The operative mortality rate of an rAAA is 40% [1, 7].

Besides the risk of rupture, patients with an AAA have an increased risk to develop cardiovascular events [1]. Cardiovascular mortality is 2.5 times higher in AAA patients than in those without an AAA [8]. In patients with a small AAA (3.0-5.4 cm), the annual risk of cardiovascular death was 3.0% (95% CI 1.7-4.3) [9]. Due to the fact that AAA patients are treated with medication to manage cardiovascular risks, it is hard to predict cardiovascular events. Therefore, a new sensitive method is needed, independent of the use of medication, to predict the risk for developing cardiovascular events.

A possible solution might be the use of the cold pressor test (CPT) in combination with an ultrasound (US) measurement, referred to as the vasomotor reactivity test (VRT), of the carotid artery (CA). This technique was developed as a non-invasive and simple method to examine the endothelial function. The endothelial cells control vascular dilation and contraction [10], which relates to future cardiovascular events [11, 12]. In healthy young individuals, the VRT causes vasodilation in the CA [13]. A pilot study showed a relation between the outcome of the VRT and the risk of cardiovascular disease, endothelial function of the coronary arteries and the ability to predict cardiovascular events in patients with peripheral artery disease [13, 14]. The risk for future cardiovascular events is increased in 4-fold in the presence of CA constriction in peripheral artery disease patients [14].

Since AAA patients are at high risk of developing cardiovascular events, the outcome of the VRT and its predictive character could be useful for this patient group. When investigating the prediction of endothelial function in combination with AAA, it is relevant to know the effect of the VRT on the abdominal aorta. It is known that the VRT causes dilation of the abdominal aorta in healthy subjects. Chandraratna et al. [15] showed that the dilation, in response to the VRT, of the abdominal aorta is 10.7 ± 4.0 % with respect to the mean baseline for non-smokers, and 3.8 ± 5.0% with respect to the mean baseline for smokers. In view of this, the CA and abdominal aorta seem to react similarly in response to sympathetic stimulation. However, correlation between the vasoreactivity of the CA and abdominal aorta has never been assessed. This could be helpful, since the VRT can be easily performed on the CA.

The goal of this project is to study the correlation between the vasoreactivity of the carotid artery and the abdominal aorta, in both healthy participants and participants with an AAA. When confirming a correlation between the vasoreactivity of the abdominal aorta and the carotid artery diameter during the sympathetic stimulation, it is expected that the endothelial function of the CA could be translated into the endothelial function of the abdominal aorta.

In addition, the influence of an AAA on the vasoreactivity will be evaluated. It is hypothesized that the abdominal aorta has a similar vasomotor response as the carotid artery, but that the presence of an AAA attenuates the vasodilation or even causes vasoconstriction. With the use of this information, future cardiovascular events could eventually be

predicted in AAA patients.

Furthermore, the method van Mil et al. [13, 14] used for determination of the vasomotor response during the VRT will be compared to a refined method that uses more parameters describing the response to the VRT. The aim is to determine which parameters can help in identifying the vasomotor response during the VRT more specifically in both the AAA patients and healthy participants, and therefore develop a refined method.

This thesis is the product of a one-year study on the correlation between the vasoreactivity of the common carotid artery (CCA) and abdominal aorta in healthy subjects and AAA patients. The outline of this thesis is as follows: The first chapter introduces all aspects of an AAA and the physiology of the VRT. In the second chapter all technical challenges and definitions are elaborated, including conventional ultrasound for the utilization during the VRT, and the traditional and refined method used in this study. The third chapter includes the RESPONSE study, which was carried out to obtain more insight into the vasoreactivity of the CCA and abdominal aorta during the VRT, in healthy young adults and AAA patients. Chapter four elaborates the spectral analysis of the measured data. In the fifth chapter the difference between measurements using the curved and linear probe is discussed. Chapter six elaborates the future perspective for the most important findings. Furthermore, a personal goal during this last year of my study was to improve my academic writing skills, which I have done by writing a case report. Hence, this thesis is concluded with a case report, about the value of IMPEDE-FX embolization plugs to embolize a false lumen of an infrarenal post-dissection aneurysm.

CHAPTER

1 Background

1.1 Abdominal aortic aneurysm

1.1.1 Background

Dilation of the abdominal aorta could eventually lead to the formation of an abdominal aortic aneurysm (AAA). An AAA is a permanent and irreversible enlargement of the abdominal aorta of at least 1.5 times the diameter of the healthy vessel or a diameter greater than 3 cm in the maximum transverse dimension [16], most commonly in a fusiform shape [17]. Most AAAs are infrarenal, which is located approximately 1 to 2 cm distal to the renal artery [18].

Risk factors for the development of an AAA include male gender, increased age, smoking, diabetes mellitus, atherosclerosis, a high level of cholesterol, hypertension and a first-degree relative with an AAA [16, 19]. Smoking was the strongest risk factor [20], and increases the risk of developing an AAA three to four times [21]. A history of hypertension involves a hazard ratio of 1.44 [20]. A unique twin registry study showed that the twin of a monozygotic twin with an AAA had a 71 times higher risk of developing an AAA than that of a monozygotic twin without an AAA [22].

1.1.2 Pathophysiology

The aorta is composed of three layers; the intima, media, and adventitia. The intima consists of a single layer endothelial cells upon loose connective tissue, while the media consists of smooth muscle cells (SMCs) in a dense matrix of fibrillar structural proteins, and the adventitia consists of fibroblasts and collagen fibers [23]. The media of the aorta provides viscoelasticity by elastin filaments connected with collagen fibers and SMCs.

An AAA occurs when the media and/or adventitia of the abdominal aortic wall becomes weakened. Four mechanisms are relevant to AAA formation: proteolytic degradation of aortic wall connective tissue, inflammation and immune responses, changed biomechanical wall stress and molecular genetics [24]. The aortic wall has heterogeneous

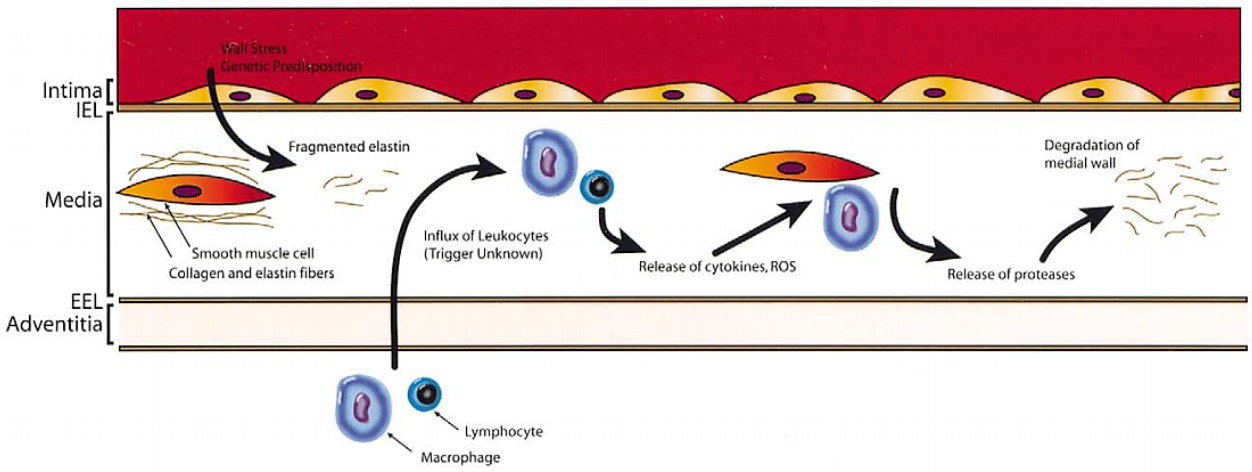


Figure 1.1: Proposed mechanism of aortic aneurysm formation, adapted from Ailawadi et al. [24]

grades of inflammation based on numbers of infiltrating T cells, macrophages and neutrophils, associated with changes in the extracellular matrix [25]. These changes include a destruction of elastin and collagen in the media and adventitia, losing SMCs which causes thinning of the wall, infiltration of lymphocytes and macrophages and neovascularization [24], which can be seen in Figure 1.1. This causes disturbed characteristics and functionality of the vasculature in these patients, like increased arterial stiffness, and changes in the response to a sympathetic stimulus [14, 26].

1.1.3 Diagnosis and treatment

AAAs are usually detected as an incidental finding when using imaging techniques for other indications [1]. The preferred imaging modality for surveillance is ultrasound (US), whereas for surgical planning a CTA is used including multiplanar and curved three dimensional reconstructions [19]. For an AAA diameter smaller than 4 cm a three year surveillance interval is safe, for AAAs with a diameter between 4.0-4.5 cm annual surveillance is safe, and an AAA diameter above 5.0 cm needs surveillance every 3 to 6 months, according to the European Society for Vascular Surgery (ESVS) guidelines [17].

If the AAA is not detected, the first symptom is rupture in many cases. Parkinson et al. [27] showed yearly rates of a ruptured AAA (rAAA) of 3.5% in AAAs of 5.5-6.0 cm, 4.1% in AAAs of 6.1-7.0 cm, and 6.3% in AAAs >7.0 cm in a systematic review. Therefore, the AAA must be detected at an early stage, return for regular follow-up and if necessary electively treated. However, the risk of death from rupture is lower than the risk of death from causes other than the AAA, mainly cardiac and pulmonary causes [27]. Therefore, treatment with cardiovascular risk management is needed, including blood pressure and lipid control as well as anti-platelet therapy and healthy lifestyle strategies (i.e. smoking cessation, exercise and diet) [19].

Most AAAs never rupture, therefore elective repair is only done when the risk of rupture is considered too high. When the AAA diameter is greater than 5.5 cm for men and 5.0 cm for women or grows more than 1 cm per year, there is an increased risk of rupture and the risks of surgery outweigh the risk of rupture [1, 5]. The risk of complications during surgery depend on age, cardiac and pulmonary function and the extent of abdominal aorta involved [16].

The two treatment options for AAAs are open surgical repair (OSR) and minimal invasive endovascular aneurysm repair (EVAR) [18]. In addition, several complex and technically challenging EVAR procedures are developed that offer the possibility to extend the proximal landing zone for stent grafts, such as fenestrated/branched EVAR or treatment with chimney grafts [19]. In the Netherlands, annually 2200 patients undergo surgery for an AAA [28]. Based on the patient's fitness and anatomy of the AAA the surgical technique will be decided.

Elective and urgent EVAR have several complications. For elective EVAR, the most common early complication is perioperative bleeding (1.9%). Hospital-acquired pneumonia is a major concern for urgent EVAR (28.5%) [29]. In a meta-analysis of survival after elective AAA repair, the five year survival rate was 69% [30]. Rupture, open repair, renal insufficiency and age are predictors of 30-day mortality [31]. The European Society Cardiology guidelines graded OSR as a high risk intervention, with a risk of cardiovascular death of 5% or more within 30 days. EVAR is graded as an intermediate risk intervention with a cardiac risk between 1-5% [19]. The advantage of perioperative survival with EVAR was sustained for three years. EVAR and OSR resulted in similar long-term survival with a hazard ratio with EVAR versus OSR of 0.97 [32]. The crude percentage of reintervention was higher for EVAR than for OSR, 16% vs 2.4%, and there was a trend towards a higher aneurysm related mortality after EVAR [33]. The most common causes of death >30 days post-AAA repair include cardiac disease (18%, atherosclerotic heart disease, acute myocardial infarction) and pulmonary disease (lung cancer (13%), respiratory failure (6%)). Causes of death after 30 days of the repair with the specific mention of aneurysm were identified in 2.4% of all deaths [34].

Cardiovascular risk

Cardiovascular risk management is of major importance in AAA patients, since they have a higher risk of death from cardiac causes than from an AAA rupture [19, 34]. Cardiovascular deaths unrelated to AAA occured in 35% of the patients in a study reported by Gonçalves et al. [31], while Brown et al. [35] reported a 49% cardiovascular mortality in the EVAR 1 trial during 5.5 years. Newman et al. [8] showed respective relative risks, after adjustment for age, risk factors and presence of other cardiovascular disease, of 1.32 for total mortality, 1.36 for cardiovascular mortality and 1.57 for incidence of cardiovascular disease in participants with AAA relative to participants without AAA. Cardiovascular mortality is 2.5 times higher in AAA patients than in those without an AAA [8]. In patients with a small AAA (3.0-5.4 cm), the annual risk of cardiovascular death was 3.0% (95% CI 1.7-4.3) [9]. All these studies indicate the high risk of cardiovascular events in AAA patients.

Since AAA patients have this high risk of cardiovascular events, the SMART (Second Manifestations of ARTerial disease) risk score is developed to determine the risk of recurrent vascular events based on clinical characteristics of

the patients [36]. AAA patients are treated with anti-platelet therapy, lipid lowering agents if lipoprotein cholesterol > 2.5 mmol/L, and antihypertensives in the case of a systolic blood pressure > 140 mmHg to manage the cardiovascular risk [19]. Five year survival rates were significantly improved for AAA patients taking statins (68% vs. 42%), antiplatelet therapy (64% vs. 40%) or antihypertensive agents (62% vs. 39%) compared with AAA patients not taking these medications [37]. Statins are also associated with reduction of AAA growth [38]. Due to the medication use, it is hard to predict cardiovascular events. The prediction of these events is of major importance, because of the high risk and mortality of cardiovascular events in AAA patients. In order to predict the risk for developing cardiovascular events, a new sensitive non-invasive method is needed.

1.2 Vasomotor reactivity test

A possible solution for the risk prediction of cardiovascular events, might be the use of the cold pressor test (CPT) in combination with ultrasound (US) measurement of the carotid artery (CA). Performing an US measurement on the CA during the CPT is called the carotid artery reactivity test (CAR-test). However, the use of the CPT in combination with US can be done on various arteries, and will therefore be referred to as the vasomotor reactivity test (VRT).

The CPT is a test that induces systemic stress. Stress exposure is associated with the activation of two systems, among which the sympatho-adrenomedullary system (SAM) [39]. The SAM triggers increased activity in the sympathetic branch of the autonomic nervous system in the case of sufficient stress, which causes release of neurotransmitters from the adrenal medulla and sympathetic nerve endings. The release of the neurotransmitters results in increasing heart rate and blood pressure, generally known as the 'fight-or-flight' response [40]. These responses are necessary for the organism's short-term survival, but if the responses are elevated for long periods of time pathological conditions may arise. Acute stress responses typically do not cause a health burden in young, healthy individuals [41]. Multiple stress induction tests have been developed whereof the CPT is a common and extensively validated test which induces systemic stress involving immersion of an individual's hand in ice water for a period of time. The CPT has been used in various fields including research examining the effects of stress on memory [42], pain [43, 44] and cardiovascular health [45–47].

It is known that temperature and other environmental stressors affect heart rate and blood pressure [48]. Cold stress as given by the CPT initiates sympathetic stimulation as described above. This stimulation is produced by the release of norepinephrine and epinephrine and an elevation in mean arterial pressure [49]. The sympathetic discharge causes peripheral vascular resistance by increasing alpha-adrenergic receptor mediated vasoconstriction [50], arteriolar constriction, increased heart rate and increased cardiac contractility, which results in an increased blood pressure [49]. Furthermore, cold may produce stimulation of the $\alpha 1$, $\alpha 2$ and β adrenergic receptors on smooth muscle cell [15]. Although Mourot et al. [51] showed that the effect on heart rate is variable, which means either an increase or remaining unchanged. It is thought to be a biphasic response with an initial increase of the heart rate in those subjects followed by a slow decline that may even return to the pretest level.

The endothelium plays a critical role in vasomotor response, and endothelial dysfunction, present in atherosclerosis, affects the vasomotor response [52]. The endothelium produces vasoconstrictor and vasodilator substances, which leads to diameter changes of the vessels via their effects on SMCs [10]. The response of the coronary arteries to CPT is suggested to be endothelium-dependent [13]. Coronary vascular response to the CPT during the VRT results in dilation in healthy subjects, equal to e.g. stimulation by infusion of acetylcholine. The common factor for vasodilation resulting from either acetylcholine or sympathetic stimulation is the endothelium, although the working mechanisms of these stimulation methods might be different.

1.2.1 Vasomotor reactivity test in atherosclerotic disease

Stress exposure to the heart may uncover latent abnormalities of myocardial contractility, e.g. angina pectoris patients may have normal ventricular function at rest but may develop abnormalities during stress [46]. The coronary artery response to sympathetic signals is altered in the presence of atherosclerosis and coronary risk factors [49, 53]. Nabel et al. [49] showed that the VRT induces constriction in atherosclerotic coronary arteries. This may be due to altered neurotransmitter sensitivity and/or endothelial dysfunction. Coronary endothelial vasodilator dysfunction predicts long-term atherosclerotic disease progression and future cardiovascular events [54]. Moreover, vasoconstriction in response of the VRT can be predictive of the prevalence and severity of long-term cardiovascular events in hypertensive patients with angiographically normal coronary arteries, while patients with a normal response remained free of cardiovascular events [55].

It is known that the CA dilates as well as the coronary arteries in response to the VRT. The CA response to a sympathetic stimulus is altered in the presence of coronary risk factors [56]. Using the VRT on the CA may be a valuable noninvasive method to assess coronary risk [56].

Furthermore, patients with peripheral arterial disease (PAD), atherosclerotic arterial stenosis and occlusions in the larger vessels of the lower extremities, have an increased risk for future cardiovascular morbidity and mortality. Carotid vasoconstriction measured using the VRT predicts cardiovascular events and progression in PAD patients, with a 4-fold increased risk in those with a vasoconstrictive response of the CA [14].

The response of the CA during the VRT, the CAR-test, functions as a strong, independent predictor of cardiovascular events in patients suffering from hypertension [14, 55, 56]. We will further explain this in section 1.2.3 'Carotid artery reactivity'.

1.2.2 Influence of age on the vasomotor reactivity test

The autonomic regulation of the cardiovascular system is affected with ageing, which depends on both neural activity and the responsiveness of its receptors [57]. Sympathetic nerve activity increases with ageing, but the mechanisms responsible for this rise are unknown. Ng et al. [58] showed that the rise in muscle sympathetic nerve activity with ageing is independent of age-related differences. The studied differences were arterial blood pressure, obesity, weight, daily energy expenditure, and ischemic heart disease. Therefore it is thought to be related to some factor associated with the ageing process per se.

Although the sympathetic nerve activity increases with ageing, the vascular response of elderly is delayed and diminished [57]. The diminished response in the elderly is suggested to be caused by the cardiac and vascular responsiveness to sympathetic nerve activity [59, 60]. It is suggested that ageing comes with an altered myogenic responsiveness, resulting in reduced contractility of the vessels to stimuli such as neurotransmitters [61, 62]. As explained earlier the sympathetic nervous system is one mechanism to regulate peripheral vascular resistance by increasing alpha-adrenergic receptor mediated vasoconstriction [50]. Sugiyama et al. [57] showed that this receptor mediated response to muscle sympathetic nerve activity may be attenuated in the elderly, which may be provoked by high concentrations of plasma norepinephrine at sympathetic nerve terminals [63].

Another mechanism that is related with the altered vasomotor response on sympathetic stimulation in the elderly is the myocardial oxygen demand. Skin surface cooling, e.g. induced by the CPT, provokes a greater increase in blood pressure and myocardial oxygen demand in older than in young adults. However, the supply of myocardial oxygen did not increase in older adults during skin surface cooling, as it did in the young [64]. This suggests that ageing comes with an altered coronary vasomotor response to acute cold stress, which may reflect disadvantageous effects of ageing on adrenergic function. Young men show vasodilation as a normal coronary vasomotor response to the VRT and this effect is lost with age as the result of a changed adrenergic mechanism [45]. The vasomotor response of the CA is also significantly larger in young than in older humans, $4.1 \pm 3.7\%$ versus $1.8 \pm 2.6\%$ respectively. Furthermore, older adults demonstrate a larger increase in heart rate and mean arterial pressure than young adults as a result of the CPT [13].

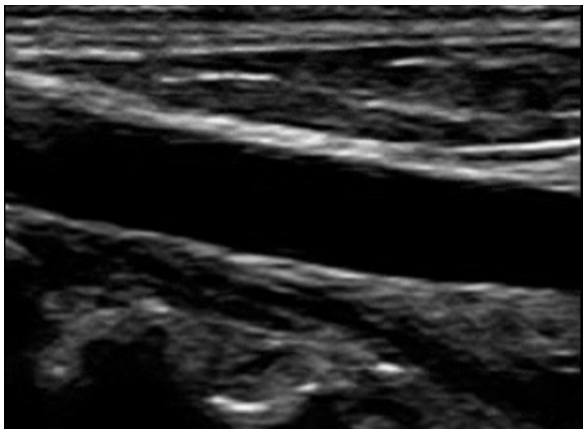
Since the AAA patients are mostly above the age of 70 years [2], it is important to take the endothelial changes and altered vasomotor response to sympathetic stimulation that come with ageing into account in this study, to make a distinction between AAA and age induced changes.

1.2.3 Carotid artery reactivity

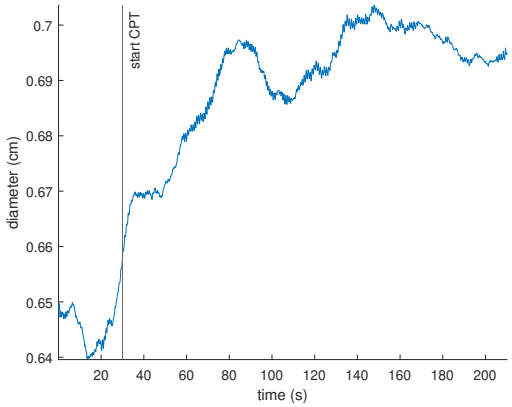
Non-invasive determination of the coronary artery diameter in order to predict long-term cardiovascular events [55] is technically challenging, expensive and lacks sufficient temporal resolution for imaging techniques to detect rapid changes in diameter [13]. However, the CA dilates as well in response to the CPT, whilst the CPT induces constriction in patients with cardiovascular disease. This response functions as a strong, independent predictor for future cardiovascular events in patients suffering from hypertension [14, 55, 56]. Therefore, carotid artery reactivity (CAR) testing was developed as a non-invasive and simple method to examine endothelial function. The CAR-test is nothing more than the VRT performed on the CA. A pilot study showed that the CAR-test has a correlation with the risk of cardiovascular diseases, to endothelial function of the coronary arteries and is able to predict future cardiovascular events in patients with peripheral artery disease [13]. During the CAR-test the CA diameter is measured in response to a sympathetic stimulus using the CPT. The CAR-test visualizes the diameter of the carotid artery using ultrasound (US) in a longitudinal section, see Figure 1.2a and 1.2b.

1.2.4 Vasomotor reactivity test and the abdominal aorta

Since AAA patients are at high risk for developing cardiovascular events, it is very relevant to know the effect and predictive value of the VRT on the abdominal aorta. It is known that the VRT shows dilation of the abdominal aorta in



(a) Screenshot from an ultrasound video of the carotid artery in the longitudinal plane.



(b) Example of the diameter change of the carotid artery induced by the CPT and measured using ultrasound.

Figure 1.2: CAR-test US measurement (a) and measured diameter change (b)

healthy subjects. The dilation of the abdominal aorta in response to the VRT is 10.7 ± 4.0 with respect to the mean baseline for non-smokers. For smokers this dilation in response to the VRT is reduced slightly to 3.8 ± 5.0% with respect to the mean baseline [15]. However, in those with impaired vascular health the dilation in response to the VRT is attenuated. In view of this, the common carotid artery (CCA) and abdominal aorta seem to react similarly in response to sympathetic stimulation. However, correlation between the vasoreactivity of the CCA and abdominal aorta has never been assessed. In addition, the vasoreactivity of the CCA and abdominal aorta has never been assessed in AAA patients.

CHAPTER

2 Technical challenges and definitions

2.1 Ultrasound specifications during the VRT

Ultrasound (US) is noninvasive, does not involve the use of ionizing radiation or contrast agents, and is relatively inexpensive [65, 66]. Modern medical US is performed mainly using a pulse-echo approach with a brightness-mode (B-mode) display [67]. In order to obtain a good quality diameter analysis during the vasomotor reactivity test (VRT), the lumen-vessel wall interface must be clearly visible on the US image. With the use of B-mode specifications accurate definition of luminal thrombus contained within the blood vessels can be obtained. Hence, the lumen-vessel wall interface visibility will be improved. This last property highly improves the diameter analysis of the US images during the VRT of the abdominal aorta and common carotid artery (CCA).

Besides the lumen-vessel wall interface visibility, the location of the artery in the longitudinal plane can improve the analysis of the diameter change during the VRT. For optimal analysis, the artery must be located perpendicular to the probe.

2.2 Analyzing the vasomotor response during the VRT: a refined method

Since the aim is to determine which parameters can help in identifying the vasomotor response during the VRT more specifically in both the AAA patients and healthy participants, the parameters were defined clearly. The newly developed parameters to describe the response to the VRT more specific are called the refined method. This refined method is compared to the traditional method, as described by van Mil et al. [13, 14]. Both methods are explained in this section.

2.2.1 Traditional method

The traditional method used BloodFlow Software (Version 5.2; National Instruments LabVIEW, Austin, TX, USA) to determine the CCA diameter. After this diameter was determined, major artefacts were deleted manually from this data set, and missing data was interpolated linearly using Microsoft Excel (Microsoft, Redmond, WA, USA). The mean baseline CCA diameter was calculated as well as the area under the curve (AUC). The vasomotor response as a result of the VRT, i.e. vasoconstriction, vasodilation and no response, was classified based on the shape of the curve with regard to the mean baseline diameter. Based on this classification, the 10-second bin with the lowest (vasoconstriction) or highest (vasodilation) diameter change was used to calculate the peak diameter change. From this peak, the time to peak could be derived, the percentage diameter change, and the diameter change with regard to the baseline was calculated.

2.2.2 Refined method

The refined method is developed to create a more objective analysis method, and describing the vasomotor response during the VRT more specifically. This method also used the BloodFlow Software to determine the CCA diameter, and major artefacts were deleted manually as well. The diameter and time data was transferred to Matlab (R2018b; The Mathworks, Inc., Natick, MA, USA). The outliers were removed using a threshold of 2.5 * Median Absolute Deviation (MAD) for the baseline measurement and CPT separately. This threshold is described by Leys et al. [68] as a reasonable threshold. Then, the missing data was filled using linear interpolation. However, if there was data missing at the first seconds of the baseline the interpolation will start from the first known value. Moreover, if there was data missing at the end of the VRT, interpolation was performed using a constant of the mean of the last 10 values. Additionally, the data

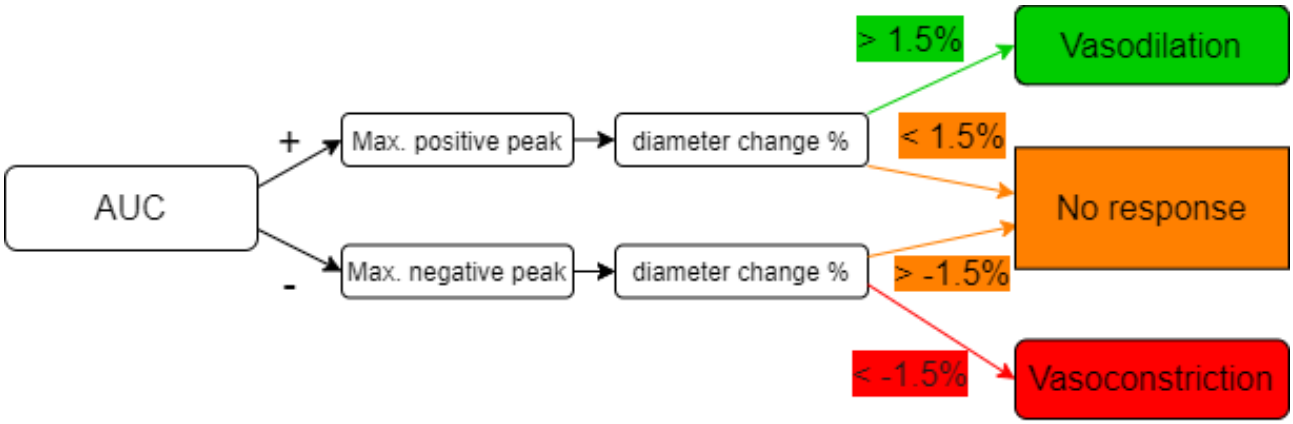


Figure 2.1: Classification steps for the determination of the vasomotor response during the VRT.

was smoothened using a moving mean filter with a 10 second time window.

The parameter definitions of the traditional method and several new parameters are listed in Table 2.1. An overview of the parameters is shown in Figure 2.2. The parameters were calculated from the diameter data after the pre-processing steps. The AUC was calculated using the trapz function in Matlab, where the diameter difference with regard to the mean baseline diameter is integrated with respect to the time. The first step of classification of the vasomotor response, i.e. vasoconstriction, vasodilation and no response, as a result of the VRT was made based on the positivity or negativity of the AUC (Figure 2.1). When the AUC was positive, the peak diameter was defined as the maximum positive value for diameter in cm. When the AUC was negative, the peak diameter was defined as the maximum negative value for diameter in cm. In the case of multiple samples with the same maximum value, the first value is defined as the peak.

The time to peak is the time from the start of the CPT to the peak. The diameter change is defined in cm and in %, both represent the change of the peak with respect to the baseline diameter. This leads to the second step of classification of the vasomotor response, where a diameter change ≥ 1.5% is called vasodilation, a diameter change ≤ -1.5% is called vasoconstriction, and a diameter change ≤ 1.5% and ≥ -1.5% is called no response (Figure 2.1). This 1.5% threshold is based on experience of the Physiology department at RadboudUMC.

The slope of the curve is calculated dividing the diameter change (in mm) by the time to peak. In addition, the duration of response is the time duration, where the diameter change is larger than 1.5%, or the time duration, where the diameter change is smaller than -1.5. Next to this parameter, the AUC of the response is calculated as the AUC when the diameter change is ≥ 1.5 in the case of vasodilation or ≤ -1.5 when vasoconstriction appears. The effective diameter change is calculated from this AUC of the response divided by the duration of the response. At last, the impact factor is the AUC of the response times the duration of response. This gives an indication of the time and strength of the response.

The duration of response, AUC of response, effective diameter change and impact factor are not calculated for the non-responders.

Table 2.1: Determined parameters of the VRT

Parameter	**Definition**
Baseline diameter (cm)	Mean diameter of the baseline
AUC (cm*s)	Diameter area under the curve from start CPT
Peak diameter (cm)	AUC >0, maximum value diameter AUC <0, minimum value diameter
Time to peak (s)	Time from start CPT to peak
Diameter change (cm)	Peak minus the mean diameter of the baseline
Diameter change (%)	Percentage change of the diameter
Slope of the curve (mm/s)	Slope from baseline diameter at start VRT to peak
Duration of response (s)	Vasodilation: Time duration of diameter change >1.5% Vasoconstriction: Time duration of diameter change <1.5%
AUC of response (cm*s)	Diameter area under the curve of the response (>1.5% or <-1.5%)
Effective diameter change (cm)	Area under the curve of the response / duration of response
Impact factor (cm*s^2)	Area under the curve of the response * duration of response

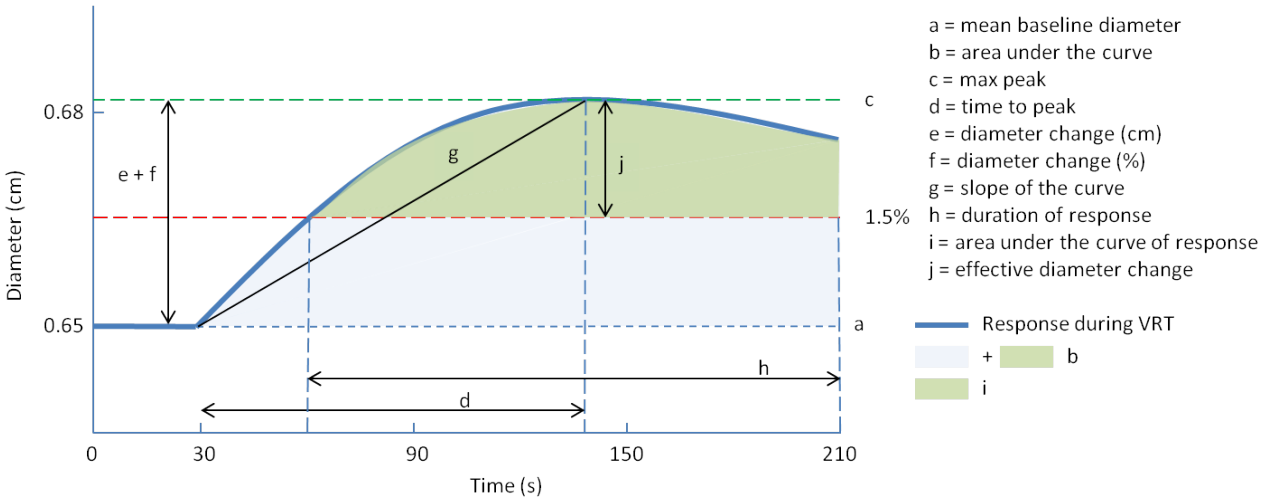


Figure 2.2: Definitions of the parameters of the refined method, where a is the mean baseline diameter, b is the AUC (the blue and green area), c is the peak diameter, d the time to peak, e and f show the diameter change, g is the slope of the curve, h is the duration of the response, i (the green area) is the AUC of the response and j is the effective diameter change

CHAPTER

3 RESPONSE study: Relation betwEen abdominal aorta and carotid artery responses to SymPathetic stimulatiON uSing duplEx ultrasound

3.1 Introduction

An abdominal aortic aneurysm (AAA) is defined as a permanent enlargement of the abdominal aorta larger than 3 cm or at least 1.5 times larger than its original diameter [16]. An AAA occurs due to a weakness in the media and/or adventitia wall of the abdominal aorta. This is characterized by destruction of elastin, collagen and smooth muscle cells, which results in thinning of the media and/or adventitia wall [24]. It can best be described as "a chronic inflammatory condition with an accompanying proteolytic imbalance" [69]. This results in disturbed characteristics and functionalities of the abdominal aorta and also the whole vasculature in these patients, like arterial stiffness and response to a sympathetic stimulus [14, 26].

If the AAA diameter increases, the risk of rupture also increases [5]. Furthermore, it is known that these patients have an increased risk in development of cardiovascular (CV) events [30]. CV mortality in AAA patients is 2.5 times higher than in those without an AAA [8]. Traditional CV risk management remains difficult, since these patients are often treated with medication to manage CV risk. This medication impairs the ability to predict future CV-events. Recent studies suggest that the reactivity of the vascular system could have a correlation with the disease development, since the whole vasculature has disturbed characteristics and functionalities.

Recently, a novel, simple, prognostic and non-invasive test of vasoreactivity was developed and validated; the carotid artery reactivity (CAR) test. The CAR test uses sympathetic stimulation by placing a hand in cold water (4 degrees Celcius) for a certain amount of time, e.g. 3 minutes, called the cold pressor test (CPT). The vasoreactivity is measured using ultrasound (US) of the carotid artery (CA) during the sympathetic stimulation. The sympathetic discharge causes peripheral vascular resistance by increasing alpha-adrenergic receptor mediated vasoconstriction [50], arteriolar constriction, increased heart rate and increased cardiac contractility, which results in an increased blood pressure [49]. Sympathetic stimulation causes strong coronary artery dilation in healthy subjects and constriction in those with cardiovascular disease (CVD), whilst this vasomotor response predicts CV-events [49, 52, 54]. Similar to coronary arteries, carotid arteries dilate during sympathetic stimulation in healthy arteries, but constrict in those with CVD [56].

Making use of this observation, the CAR test was developed. This simple and robust test is practical and technically feasible [56]. Pilot work showed that the CAR test relates to CVD risk, to coronary artery endothelial function and predicts CV-events in peripheral artery disease patients [13]. However, the use of the CPT in combination with US measurements of the arterial diameter can be done on various arteries next to the carotid artery, and will therefore be referred to as the vasomotor reactivity test (VRT).

Literature has also demonstrated the effect of the VRT on the abdominal aortic diameter [15]. The VRT shows dilation of the abdominal aorta in healthy subjects, whilst this is attenuated in those with impaired vascular health [15]. This suggests that the vasomotor response throughout the whole vascular system is approximately the same. However, correlation between the vasoreactivity of the CA and abdominal aorta is not proven yet. It is hypothesized that the abdominal aorta has the same functional response as the common carotid artery (CCA), but that an AAA attenuates or even reverses this response for both the CCA and abdominal aorta.

This study aims to develop a refined method for the examination of the vasomotor response, which will be compared to the traditional method as executed by van Mil et al. [13, 14], since an objective method for this is needed. The refined method is expected to be similar to, but more objective than, the traditional method. Secondly, the vasomotor response induced by the VRT of the abdominal aorta will be compared to the response of the CCA. The correlation is expected to be good in healthy young subjects, because the same functional response to the VRT is expected. When looking at both

healthy young subjects and AAA patients, independent of age and the presence of an AAA, the correlation is expected to improve by increasing the sample size. Finally, the vasomotor response of healthy young subjects will be compared to the response in AAA patients. When different responses are measured for the AAA patients, this gives confidence for the development of a predictive model for CV events in AAA patients.

3.2 Method

3.2.1 Study population

The study is ongoing while not all subjects are included yet, and the interim results are presented. A total of 31 subjects is included divided over 2 groups: healthy young adults, and patients with an untreated AAA. The healthy young adults, between the age of 18 and 40 years old, were recruited through personal contacts, flyers and from databases of participants from previous studies at the department of Physiology of Radboudumc and the department of Vascular Surgery of Rijnstate who have given permission to contact them for future studies. Patients with an AAA were recruited from the Rijnstate hospital.

The AAA patients meet the following inclusion criteria: an AAA under surveillance with a diameter between 3.0 and 5.0 cm. Exclusion criteria for both groups were a psychiatric or other condition that may interfere with the study, participation in another clinical study interfering on outcomes, a BMI ≥ 30 kg/m^2, an increased risk for coronary spasms (score Rose-questionnaire ≥ 2, Appendix 8.1), known carotid artery disease, the presence of Raynaud's phenomenon, Marfan syndrome, chronic pain syndrome in the upper extremity(s), presence of an AV fistula or shunt, open wounds to the upper extremity(s), and/or scleroderma associated with placing the hand in ice water, and the recent (<3 months) presence of angina pectoris, myocardial infarction, cerebral infarction, and/or heart failure, or PAD treatment. In addition, the healthy subjects were excluded when meeting one of the following criteria: systolic blood pressure (BP) > 140 and/or diastolic BP > 90 mmHg, cardiovascular history and/or anti-hypertensive medication.

Written informed consent was obtained from all participants prior to participation. Ethical approval was obtained from the local Ethics committee with number 2019-5560.

3.2.2 Experimental design

All participants were planned for a single visit of approximately 30 minutes. The healthy young patients were measured at the department of Physiology of Radboudumc, and the AAA patients were measured at the department of Vascular Surgery of the Rijnstate hospital. Participants were asked to abstain from strenuous exercise for 24h, fast for at least 6h and they were given instructions with regard to food and fluid intake known to alter endothelial function (i.e. caffeine, alcohol and vitamin C) for at least 18h prior to the testing sessions, according to a physiological guideline [70–76]. Participant characteristics were registered, including traditional risk factors and CV history. All individuals underwent the VRT, involving simultaneous US measurements of the CCA and abdominal aorta diameter at 30 seconds baseline and the CPT, a three minute exposure of one hand into ice water.

The different aims of this study are described in Figure 3.1. For aim 1 (i.e. comparison of the traditional method and the refined method, as described in section 2.2), the CCA data of young healthy subjects (n=20) was used. The parameters of the traditional method were compared to these parameters calculated using the refined method. For aim 2a and b (i.e. relation between the abdominal aorta and the CCA), both arteries in the young healthy subjects (2a, n=20) were studied, and in the young healthy subjects and the AAA patients (2b, n=31), using the refined method. Aim 3 (i.e. difference between healthy subjects and AAA patients) studied the healthy young subjects (n=20) and the AAA patients (n=11) using both arteries and the refined method.

3.2.3 Measurement procedure

After the participant rested for 10 minutes in supine position, the BP was measured twice (T=0). A curved probe was placed right above the aortic bifurcation and an optimal image had to be found (where the vessel wall of the abdominal aorta was clearly visible). The probe was held stable and US parameters were set to further improve the visibility of the lumen-arterial wall interface using a longitudinal, B-mode image of the abdominal aorta. At Radboudumc the 52CA probe was used in combination with a Terason T3300 device (Terason, Burlington, MA, USA) probe was used, and at Rijnstate the C5-1 probe (Philips, Amsterdam, the Netherlands) was used. Participants were positioned with the neck extended to allow assessment of the CCA. The CCA diameter was measured using a linear probe, at Radboudumc the L14-5 probe (Ultrasonix, Richmond, Canada) was used and at Rijnstate the UST-5548 probe (Hitachi Aloka medical, Twinsburg, OH, USA) was used. The US measurement of the CCA was performed at the left side, unless only the right side could be measured for a particular reason.

The VRT consisted of a baseline measurement (30 sec) and the exposure of one hand into ice water (3 min). The US measurement of both the abdominal aorta and CCA were performed simultaneously for the entire duration of

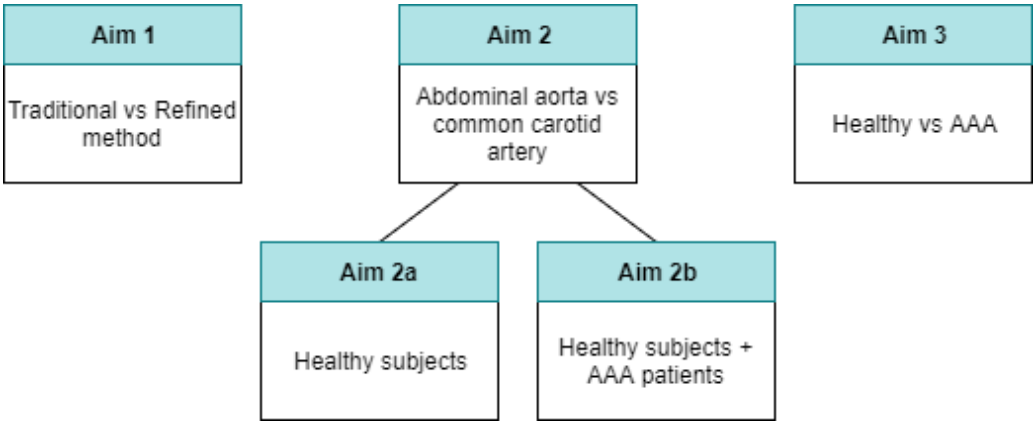


Figure 3.1: Overview to provide insight into the different methods and subgroups to answer the three aims.

the VRT. The temperature of the ice water was ≤ 4 degrees Celcius for all participants. The BP was measured every 60 seconds, after emerging the hand in ice water (T=1, 2 and 3). All BPs were measured at the right arm, using the Connex Spot sphygmomanometer (Welch Allyn, Skaneateles Falls, NY, USA) at Rijnstate Hospital, and the automatic sphygmomanometer (Omron Healthcare, Kyoto, Japan) at Radboudumc.

3.2.4 Data analysis

The measured data was saved as an Audio Video Interleave (AVI) file. The AVI files were loaded into BloodFlow Software (Version 5.2; National Instruments LabVIEW, Austin, TX, USA). This software uses a special developed edge-detection and wall-tracking algorithm in order to determine the diameter of the vessel lumen. The exact algorithm is unknown. The settings of the software were set at the Advanced Edge analysis method, using a line thickness of 2. A region-of-interest (ROI) of the artery was selected where the lumen-arterial wall interface was clearly visible. Of this ROI, the diameter was determined multiple times per frame, where the number of detected segments of the vessel wall depended on the size of the ROI. Every frame gives a median diameter, that can be used for further analysis. This data was manually filtered on major artefacts, e.g. caused by swallowing, and probe movement. The diameter and time data was transferred to Matlab (R2018b; The Mathworks, Inc., Natick, MA, USA). First, the outliers were removed using a threshold of 2.5 * Median Absolute Deviation (MAD) for the baseline and CPT separately. Then, the missing data was filled using linear interpolation. However, if there was data missing at the first seconds of the baseline this interpolation started from the first known value. Moreover, if there was data missing at the end of the CPT, interpolation was performed using a constant of the mean of the last 10 values. Additionally, a moving mean of the data was calculated using a 10 second time window. From this moving mean the baseline diameter, diameter AUC, peak diameter, time to peak, diameter change (cm), diameter change (%), slope of the curve, duration of response, AUC of response, effective diameter change and the impact factor were calculated, as described in section 2.2.2. The classification of the response during the VRT is also done as described in section 2.2.2. A maximum diameter change between -1.5 and 1.5% was considered as a non-response, based on experience at the Radboudumc.

3.2.5 Statistical analysis

All data were presented as median and the interquartile range (IQR) unless stated otherwise. Statistical analysis was performed using IBM SPSS Statistics 25 (IBM SPSS, IBM Corp., Armonk, NY, USA). The baseline characteristics and vasomotor response data of both groups were tested for normal distribution using the Kolmogorov-Smirnov tests of normality. Independent student's t-tests were used when data were normally distributed and Mann-Whitney U tests were used when data were not normally distributed. The statistical significance threshold was set at $p<0.05$. For aim 1, a Bland-Altman plot was made for all traditional method parameters, to explore the difference between the traditional and refined method. For aim 2a, Pearson's correlation coefficient (PCC) was calculated per parameter of the refined method to study the relation between the vasomotor response in the carotid artery and the abdominal aorta in the healthy young subject group. For aim 2b, the same was done as for aim 2a, but the data of both the healthy young subjects and the AAA patients was used. Aim 3 uses the independent student's t-tests or Mann-Whitney U tests to study the difference per parameter between the healthy young subjects and AAA patients. This was done for the CCA and the abdominal aorta.

3.3 Results

Twenty subjects were included in the young healthy group, 12 male and 8 female. In the AAA group 11 male patients were included. 9 aneurysms where fusiform in shape and 2 of the aneurysms were saccular, all non-inflammatory. The patient characteristics (Table 3.1) did not have a normal distribution based on the one-sample Kolmogorov-Smirnov test. Age, BMI, systolic BP, diastolic BP, CCA diameter, and abdominal aortic diameter were significantly different in the young healthy group and the AAA group.

The data of the abdominal aorta of two AAA patients could not be used, due to extensive thrombotic plaque formation, disturbing the wall-tracking algorithm, and low quality US measurement. The percentage of removed outliers has a mean of 17 ± 10% of the total data in the refined method.

Table 3.1: Mean and standard deviation of patient characteristics of both groups. P-values based on the Mann-Whitney U test.

	Young healthy group (n=20)	AAA group (n=11)	P-values
Age (years)	24 [21,26]	69 [68,77]	<0.001
Height (cm)	178 [176,182]	177 [173,180]	0.495
Weight (kg)	72 [70,79]	81 [70,87]	0.247
BMI (kg/m^2)	22.76 [21.94,24.57]	24.62 [23.98,26.39]	0.007
Systolic BP (mmHg)	124 [115,134]	150 [133,157]	0.002
Diastolic BP (mmHg)	66 [63,71]	84 [78,89]	<0.001
Heart Rate (beats/min)	61 [58,67]	65 [61,70]	0.408
Carotid artery diameter (cm)	0.63 [0.61,0.68]	0.76 [0.73,0.83]	<0.001
Abdominal aortic diameter (cm)	1.46 [1.35,1.65]	3.75 [3.27,3.95]	<0.001

3.3.1 Aim 1: Traditional vs refined method

Maximum diameter change (%), time to peak and AUC are determined for the CCA of the young healthy subjects (n=20) using the traditional and refined method. The classification of response during the VRT based on the maximum diameter change was different for two subjects.

The differences between the traditional and refined method were normal distributed for all parameters, and therefore Bland-Altman plots could be used for this analysis. The Bland-Altman plots (Figure 3.2, 3.3 and 3.4) show the differences in maximum diameter change, time to peak and AUC between the traditional and refined method. There are three outliers in the maximum diameter change, of which two have a different classification for the two methods. The average discrepancy between methods is 0.25% for this parameter, and the limits of agreement are -4.1 and 4.5%.

The Bland-Altman plot for time to peak shows one major outlier, and the average discrepancy between methods is 8.7 seconds. The limits of agreement are -47 and 64 seconds.

The plot for AUC shows three outliers, of which one has a disagreement in the positivity/negativity of the AUC. The average discrepancy between methods is -0.23 cm*s, and the limits of agreement are -2.2 and 1.7 cm*s.

3.3.2 Aim 2: Abdominal aorta vs common carotid artery

All parameters and PCCs have been calculated for the CCA and abdominal aorta of the healthy young group (n=20) and the AAA patients (n=11), using the refined method.

Aim 2a: healthy subjects

Table 3.2 shows the median and IQR of the parameters of the CCA and the abdominal aorta and their PCC. All parameters show a positive relation, except for the time to peak and the duration of response. The time to peak of the abdominal aorta is 1.8 times the time to peak of the CCA, and the duration of response is 1.6 times higher for the abdominal aorta. The mean baseline diameter has the highest correlation (r=0.51) between both arteries.

The maximum diameter change is 3.5% [-1.8,5.7] for the CCA and 4.9% [2.5,8.2] for the abdominal aorta. Figure 3.5 shows the maximum diameter change (%) for the CCA and abdominal aorta. Fourteen of the subjects showed a vasodilation of both the CCA and the abdominal aorta, three subjects have a vasodilation of the abdominal aorta and a vasoconstriction of the CCA, two subjects have a vasoconstriction of both arteries, and one subject showed a vasoconstriction of the CCA and no response of the abdominal aorta.

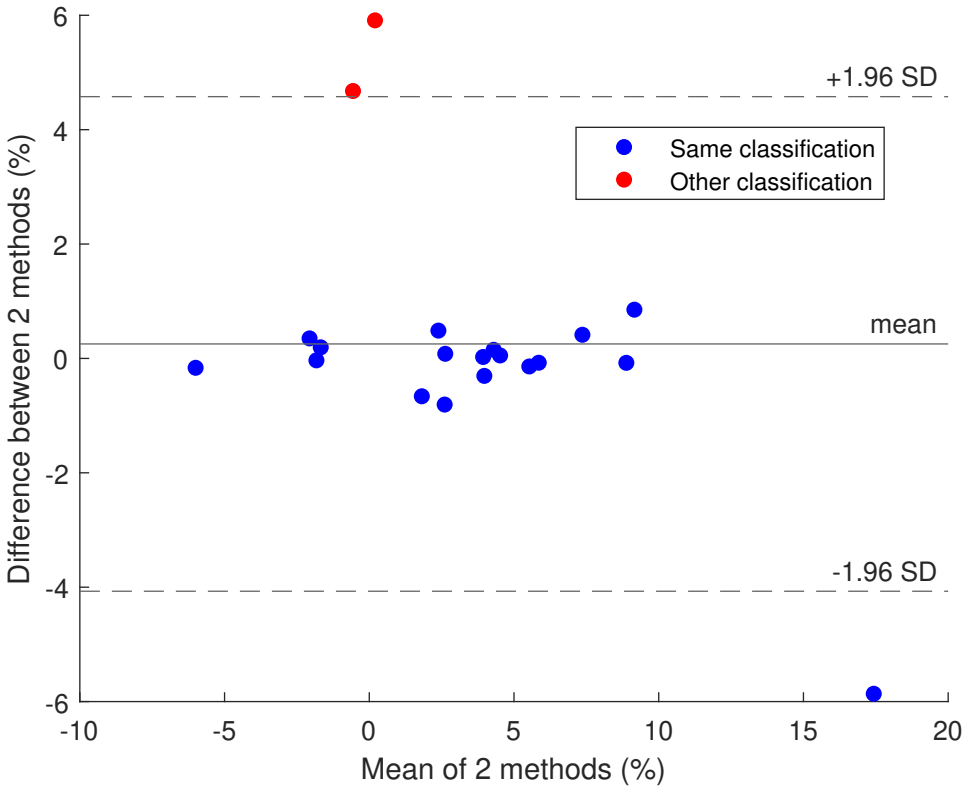


Figure 3.2: Bland Altman plot of the maximum diameter change difference measured by the traditional and refined method

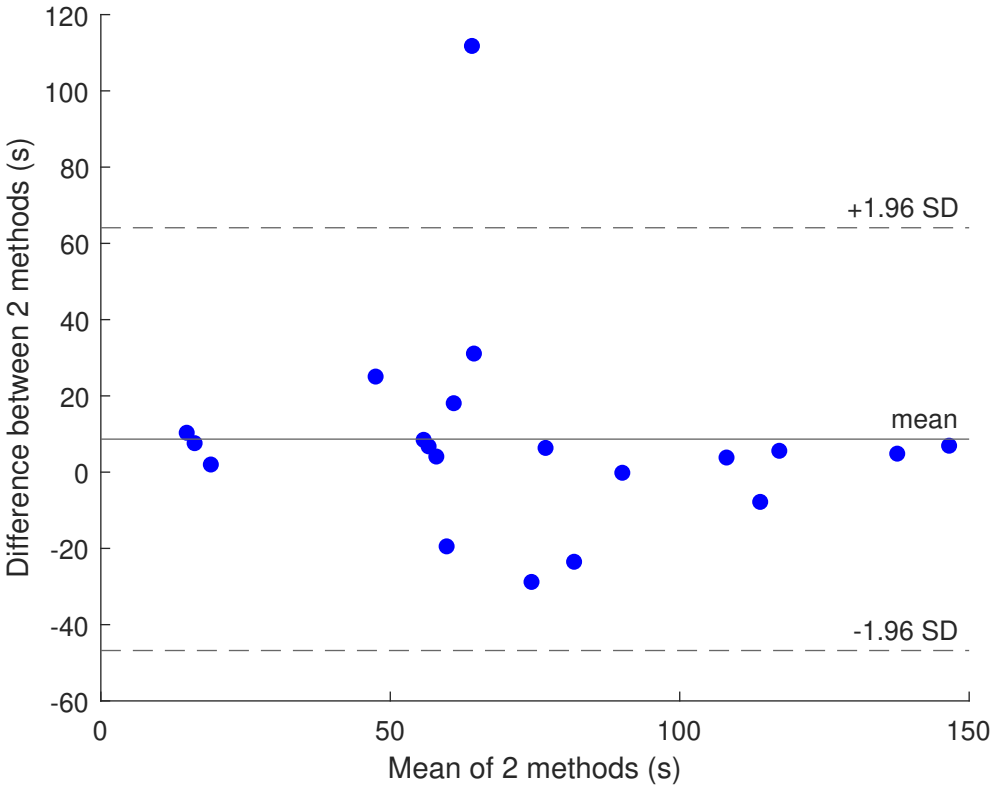


Figure 3.3: Bland Altman plot of the time to peak measured by the traditional and refined method

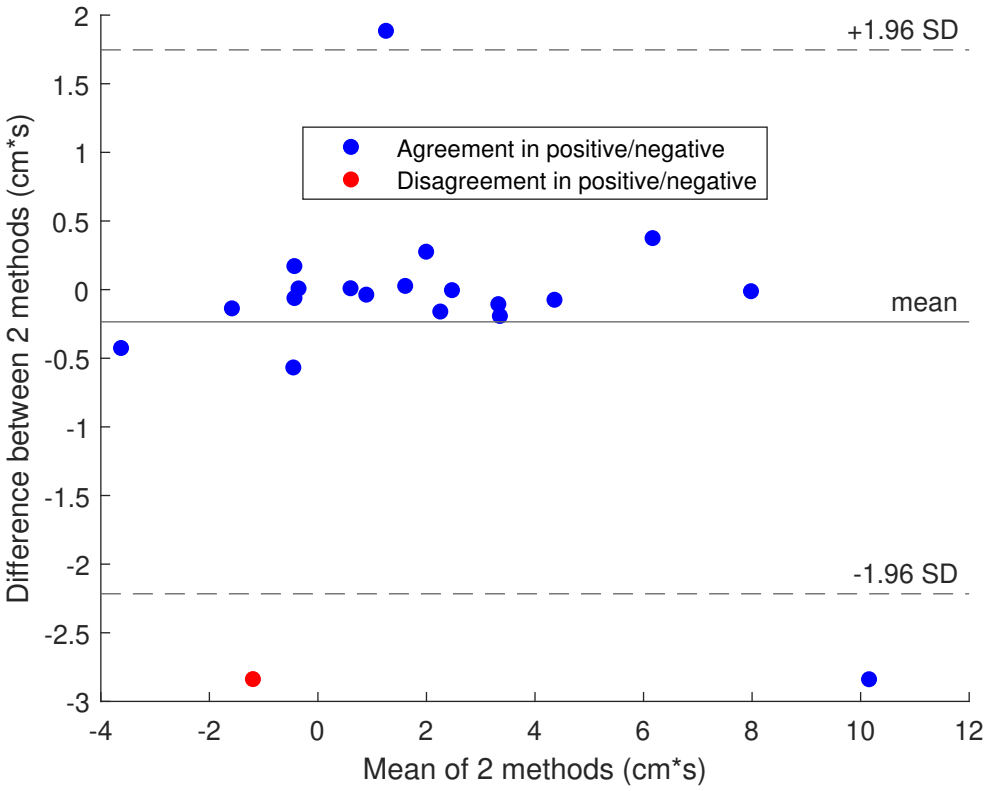


Figure 3.4: Bland Altman plot of the AUC measured by the traditional and refined method

Table 3.2: Median [Q1,Q3] of the parameters defined by the refined method for the CCA and abdominal aorta of the healthy young patient group, and their PCC.

Parameters	CCA	Abdominal aorta	PCC
Mean baseline diameter (cm)	0.63 [0.62,0.68]	1.46 [1.36,1.65]	0.51
AUC (cm*s)	1.25 [-0.22,3.40]	5.77 [0.84,13.40]	0.31
Max diameter change (%)	3.46 [-1.78,5.67]	4.91 [2.50,8.23]	0.34
Max peak (cm)	0.67 [0.62,0.71]	1.51 [1.40,1.73]	0.35
Max diameter change (cm)	0.02 [-0.01,0.04]	0.07 [0.04,0.12]	0.35
Time to peak (s)	62.69 [45.41,96.67]	119.52 [94.74,169.84]	-0.32
Slope (mm/s)	$3.26 * 10^{-3}$ [$-2.97 * 10^{-3}$, $4.39 * 10^{-3}$]	$6.19 * 10^{-3}$ [$3.09 * 10^{-3}$, $* 11.43\ 10^{-3}$]	0.21
Duration of response (s)	83.45 [32.02,147.81]	132.45 [79.34,173.46]	0.000
AUC of response (cm*s)	0.30 [0.00,1.88]	2.19 [0.79,9.78]	0.18
Effective diameter change (cm)	$4.86 *10^{-3}$ [$-0.95 *10^{-3}$, $12.14 *10^{-3}$]	$18.79 *10^{-3}$ [$10.23 *10^{-3}$,$64.87 *10^{-3}$]	0.21
Impact factor (cm*s^2)	24.04 [-0.01,266.68]	305.71 [59.88,1316.78]	0.15

Aim 2b: healthy subjects + AAA patients

Table 3.3 shows the PCC of the healthy young subjects and the PCC of the healthy young subjects and the AAA patients (both groups). When taking both groups together, the positive correlation is higher for the mean baseline diameter, max diameter change (%), max peak, AUC of response, effective diameter change and impact factor. The correlations of the other parameters become lower.

3.3.3 Aim 3: Healthy subjects vs AAA patients

All parameters from the refined method have been calculated for the CCA (n=11) and abdominal aorta (n=9) of the AAA group. These were compared to the parameters of the young healthy group.

In Figure 3.6 the maximum diameter change (%) can be seen for the CCA and abdominal aorta, for both the young healthy subjects and the AAA patients. The median of the maximum diameter change for the abdominal aorta is 4.9% [2.5,8.2] in the young healthy group, and -2.3% [-3.7,-0.7] in the AAA group. In the AAA group, only 1 patient shows vasodilation in both arteries, whereas there are 14 healthy subjects in this quadrant of the figure. One patient has a

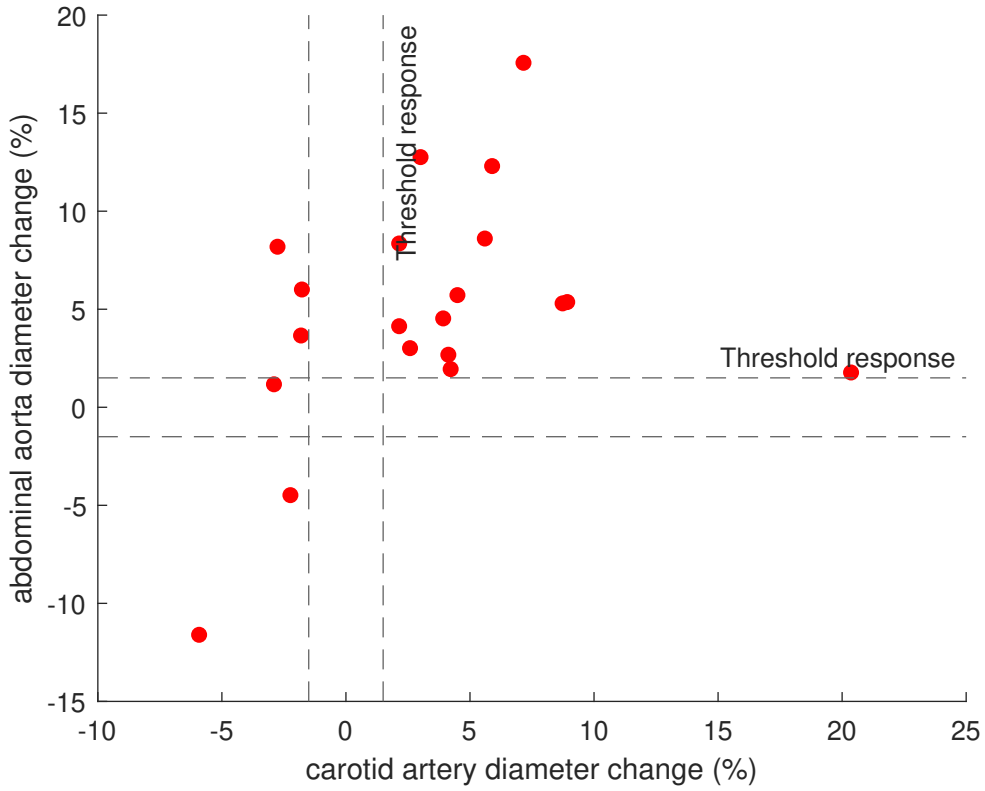


Figure 3.5: Scatterplot of the maximum diameter change (%) of the carotid artery and the abdominal aorta for the young healthy subjects (n=20), where every red dot represents one subject. The dashed line represents the threshold for the response, which is set at 1.5% diameter change for both the carotid artery and the abdominal aorta.

Table 3.3: PCC of the healthy young subjects and the PCC of the healthy young subjects and the AAA patients (both groups) for all parameters.

Parameters	PCC healthy young subjects	PCC both groups
Mean baseline diameter (cm)	0.51	0.80
AUC (cm*s)	0.31	0.31
Max diameter change (%)	0.34	0.40
Max peak (cm)	0.35	0.75
Max diameter change (cm)	0.35	0.33
Time to peak (s)	-0.32	-0.26
Slope (mm/s)	0.21	0.12
Duration of response (s)	0.000	0.12
AUC of response (cm*s)	0.18	0.23
Effective diameter change (cm)	0.21	0.25
Impact factor (cm*s^2)	0.15	0.21

vasodilation of the abdominal aorta and a vasoconstriction of the CCA, opposing 3 healthy subjects. Three patients show vasoconstriction in both arteries, opposing 2 healthy subjects. A vasodilation of the CCA and a vasoconstriction in the abdominal aorta is present in 2 AAA patients, which does not occur in the healthy group. Two AAA patients show no response in the abdominal aorta, while one of them has a vasodilation in the CCA.

In Table 3.4, the abdominal parameters of the healthy subjects are compared with the abdominal parameters of the AAA patients. A Mann-Whitney U test was performed, since the data was not normal distributed. The mean baseline diameter, AUC, maximum diameter change (%), maximum peak, maximum diameter change (cm), slope, AUC of response, effective diameter change, and impact factor were significantly different for the two groups.

In Table 3.5, the carotid parameters of the healthy subjects are compared with the carotid parameters of the AAA patients. A Mann-Whitney U test was performed, since the data was not normal distributed. The mean baseline diameter,

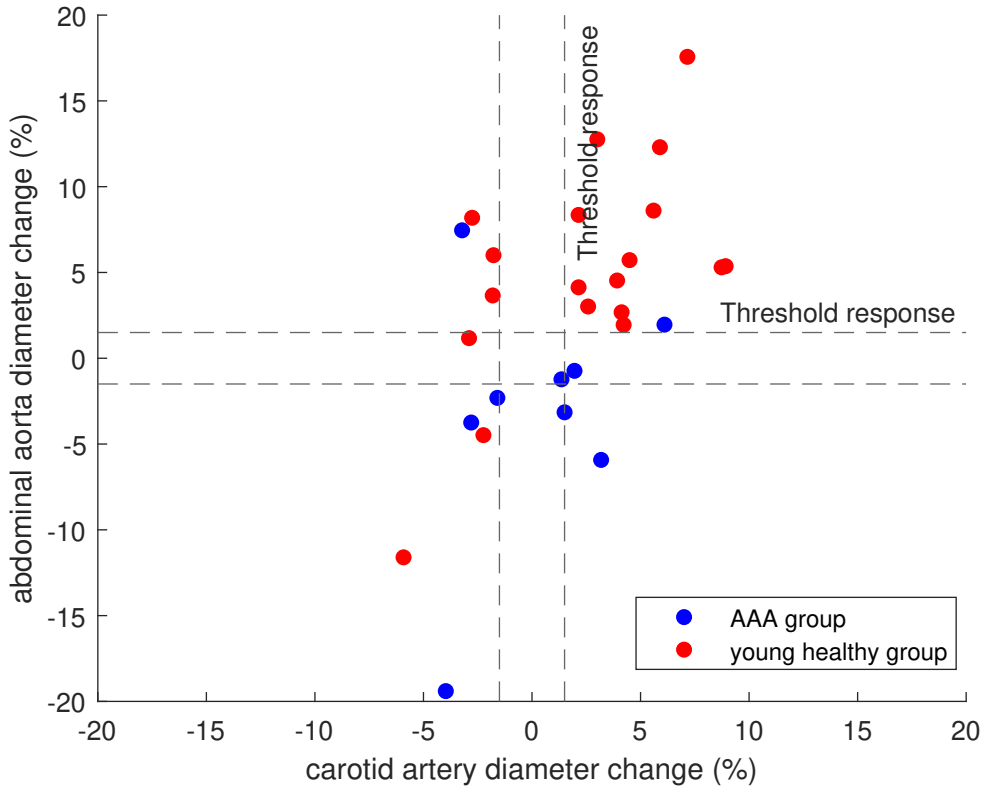


Figure 3.6: Scatterplot of the maximum diameter change (%) of the carotid artery and the abdominal aorta for the young healthy subjects (n=20) and the AAA patients (n=9). Every blue dot represents an AAA patient and every red dot represents one young healthy subject. The dashed line represents the threshold for the response, which is set at 1.5% diameter change for both the carotid artery and the abdominal aorta.

Table 3.4: Median [Q1,Q3] of the abdominal aortic parameters of the healthy subjects and AAA group, and their p-value based on the Mann-Whitney U test.

Parameters	Healthy aorta	AAA	P-value
Mean baseline diameter (cm)	1.46 [1.36,1.65]	3.87 [3.72,3.99]	<0.001
AUC (cm*s)	5.77 [0.84,13.40]	-4.42 [-12.25,-0.15]	0.020
Max diameter change (%)	4.91 [2.50,8.23]	-2.31 [-3.75,-0.73]	0.004
Max peak (cm)	1.51 [1.40,1.73]	3.83 [3.45,4.00]	<0.001
Max diameter change (cm)	0.07 [0.04,0.12]	-0.07 [-0.15,-0.03]	0.010
Time to peak (s)	119.52 [94.74,169.84]	119.80 [89.88,138.34]	0.945
Slope (mm/s)	$6.19 * 10^{-3}$ [$3.09 * 10^{-3}$, $11.43 * 10^{-3}$]	$5.93 * 10^{-3}$ [$-11.01 * 10^{-3}$, $2.48 * 10^{-3}$]	0.018
Duration of response (s)	132.45 [79.34,173.46]	117.24 [76.08,153.53]	0.651
AUC of response (cm*s)	2.19 [0.79,9.78]	-2.58 [-6.64,-0.04]	0.035
Effective diameter change (cm)	$18.79 * 10^{-3}$ [$10.23 * 10^{-3}$,$64.87 * 10^{-3}$]	$-27.20 * 10^{-3}$ [$-56.32 * 10^{-3}$,$-1.62 * 10^{-3}$]	0.025
Impact factor (cm*s^2)	305.71 [59.88,1316.78]	-225.02 [-801.19,1.06]	0.035

maximum peak, and time to peak were significantly different for the two groups. The median of the maximum diameter change for the CCA is 3.5% [-1.8,5.7] in the young healthy group, and 1.4% [-3.0,2.2] in the AAA group.

The young healthy group showed more patients classified with vasodilation than the AAA group for both arteries, as can been seen in Figure 3.7. As a result, the young healthy group showed less vasoconstriction and no response than the AAA patients. For the AAA group the percentages of vasodilation and vasoconstriction were equal in the CCA, whereas the percentage of vasoconstriction was slightly higher than the vasodilation in the abdominal aorta.

Table 3.5: Median [Q1,Q3] of the carotid parameters of the healthy subjects and AAA group, and their p-value based on the Mann-Whitney U test.

Parameters	Healthy	AAA	P-value
Mean baseline diameter (cm)	0.63 [0.62,0.68]	0.76 [0.73,0.83]	<0.001
AUC (cm*s)	1.25 [-0.22,3.40]	0.60 [-1.61,1.46]	0.169
Max diameter change (%)	3.46 [-1.78,5.67]	1.36 [-3.01,2.16]	0.054
Max peak (cm)	0.67 [0.62,0.71]	0.78 [0.75,0.82]	<0.001
Max diameter change (cm)	0.02 [-0.01,0.04]	0.01 [-0.03,0.02]	0.054
Time to peak (s)	62.69 [45.41,96.67]	124.25 [99.50,152.24]	0.003
Slope (mm/s)	$3.26 * 10^{-3}$ [$-2.97 * 10^{-3}$, $4.39 * 10^{-3}$]	$0.98 * 10^{-3}$ [$-1.95 * 10^{-3}$,$1.32 * 10^{-3}$]	0.169
Duration of response (s)	83.45 [32.02,147.81]	74.12 [38.51,116.19]	0.502
AUC of response (cm*s)	0.30 [0.00,1.88]	0.00 [-0.50,0.11]	0.091
Effective diameter change (cm)	$4.86 *10^{-3}$ [$-0.95 *10^{-3}$, $12.14 *10^{-3}$]	$-0.18 *10^{-3}$ [$-6.64 *10^{-3}$,$2.38*10^{-3}$]	0.055
Impact factor (cm*s^2)	24.04 [-0.01,266.68]	0.00 [-36.87,4.85]	0.091

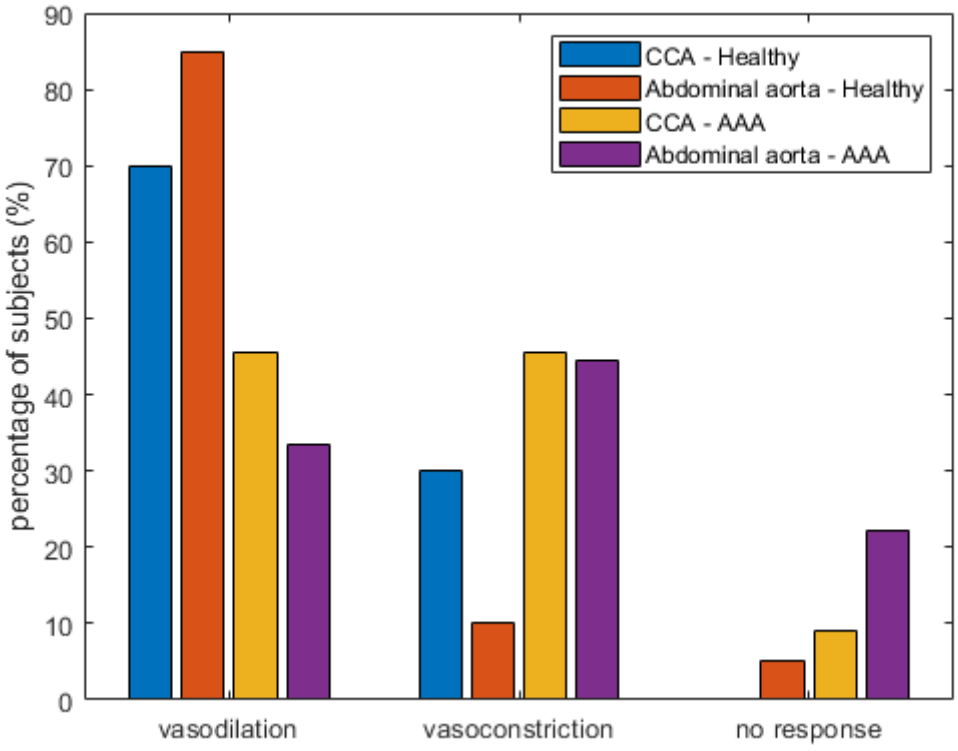


Figure 3.7: Bar graph of the classification percentages of the response during the VRT: vasodilation, vasoconstriction and no response. The bar graphs show the CCA (blue) and the abdominal aorta (orange) of the healthy subjects and the CCA (yellow) and the abdominal aorta (purple) of the AAA patients.

3.4 Discussion

3.4.1 Traditional vs refined method

In this study, a refined method for examination of the vasomotor response in comparison with the traditional method as executed by van Mil et al. [13, 14] is explored. It was found that the difference between the traditional and refined method for the maximum diameter change, time to peak and AUC is between the limits of agreement for the majority of the subjects. This suggest that the determined parameters are equal for both the traditional and refined method, which gives confidence for the use of the refined method with regard to the examination of the vasomotor response.

The traditional and refined method did not show the exact same outcome for the maximum diameter change, time to peak and AUC of the healthy young subjects. The limits of agreement are wide for all parameters, which means that the data is ambiguous. However, the average discrepancy for maximum diameter change is 0.25% between the two methods, while the median of the maximum diameter change is 3.5% [-1.8,5.7] for the CCA of all healthy young

subjects. Therefore, the discrepancy of 0.25% is considered small, and the effect on the outcome can be neglected. For the time to peak the average discrepancy is 8.7 seconds, while the median is 63 s [45,97]. Again, the error is small and will therefore not contribute significantly to the outcome. The AUC has a discrepancy of -0.23 cm*s, with a median of 1.252 cm*s [-0.218,3.395]. This discrepancy is slightly higher than the discrepancy of the other parameters, but still fits into the limits of agreement and will not affect the outcome. This suggests that the refined method is applicable for examining the vasomotor response objectively.

However, the classification of the vasomotor response is of great importance in the evaluation of the refined method, since this will eventually help in predicting future cardiovascular events. For the diameter change parameter there were two patients with different classifications. The refined method defined a negative diameter change for one of the patients based on a negative AUC, whereas the traditional method defined a positive diameter change based on the shape of the curve with regard to the mean baseline. The refined method is preferred in this case, since it used an objective and mainly automated method for this determination. The other patient with another classification for diameter change is based on the mean baseline diameter. For the traditional method a baseline from 30 to 35 seconds was chosen, while for the refined method the baseline from 0 to 30 seconds was used. The same baseline should be used in order to be able to compare the values. Therefore, it is recommended to use the same baseline in future research. The disagreement in the positive and negative AUC parameter is caused by the used filtering in the refined method. Outliers were removed, which resulted in a smoother signal than the curve of the traditional method. In conclusion, the lack of a gold standard for this classification of vasomotor response induced by the VRT complicates the development of an objective method, but at the same time emphasizes the need of an objective method. However, since the differences between the methods are so small and can be evaluated and explained by exploring the data, there is confidence for the refined method and its new parameters for describing the vasomotor response.

3.4.2 Abdominal aorta vs common carotid artery

The second aim of this study was to compare the vasomotor response measured using the VRT of the abdominal aorta to the response of the CCA in healthy young subjects. A positive correlation was found for the mean baseline diameter of both arteries of r=0.51. The other parameters had slightly lower to poor positive correlations, varying from r=0.00 to r=0.35, except for the time to peak which showed a negative correlation (r=-0.32). These correlations are lower than expected, and might suggest that the vasomotor response of the abdominal aorta deviates from the vasomotor response of the CCA. However, when determining the correlation for the healthy young subjects and AAA patients together, the correlation for mean baseline diameter, max diameter change (%), max peak, AUC response, duration response, effective diameter change and impact factor increased, varying from r=0.12 to r=0.80. This suggests that the correlation improved, independent from age and vascular disease. When more data is available this correlation might improve even more. Van Mil et al. [13] found a correlation of r=0.486 for the CA and coronary arteries, which was stated as a significant positive correlation. Therefore, the correlation of the max diameter change (%) for both groups (r=0.40) is quite good, and might improve when adding more data.

This study was the first study to compare the CCA to the abdominal aorta during the VRT, and the first study to measure the VRT in AAA patients as well. While performing the VRT on the abdominal aorta, this study found a vasomotor response induced by the CPT. In large arteries, e.g. the brachial and carotid artery, significant changes have been shown in the vessel wall mechanics after changes in activation of the SMCs [77, 78]. Chandraratna et al. [15] conducted histological studies that revealed the abdominal aorta is predominantly a muscular artery. The media of the abdominal aorta has a few elastic fibers, but is mainly composed of SMCs. This would mean that the vessel wall mechanics of the abdominal aorta would change as well after changes in activation of the SMCs, which is confirmed with the measured vasomotor response of the abdominal aorta in this study.

Gerová et al. [79] showed that sympathetic stimulation results in an increase of BP, accompanied by an increase of the radius of the aorta and a thinning of the aorta wall by using platinum electrodes on the sympathetic trunk of dogs. A stabilized blood pressure was associated with a decrease of radius and wall tension, while the aortic wall thickened. In vitro studies did not show a significant response of the abdominal aorta on sympathetic stimulation, and therefore predicted that in vivo measurements in human would not show a response of the abdominal aorta [80]. Sonesson et al. [80] stated that sympathetic stimulation by lower body negative pressure does not change the aortic diameter in humans. However, in this (RESPONSE) study a vasodilation is found in the abdominal aorta in response to the CPT in young healthy patients with a median diameter change of 4.9% [2.5,8.2]. This finding is consistent with that of Chandraratna et al. [15] who showed a vasodilation of the abdominal aorta in young healthy patients in response to the CPT. In this study, a diameter change of 10.7 ± 4.0% was found in non-smokers, and 3.8 ± 5.0% in smokers. The diameter change in the present study lies between these two values. This confirms the expectations, since the young healthy group of the present study includes both smokers and non-smokers.

The correlation between the parameters of the abdominal aorta and the CCA of the young healthy subjects were lower than expected. However, overall the CCA and abdominal aorta responded to the VRT with vasodilation, with a diameter change of 3.5% [-1.8,5.7] and 4.9% [2.5,8.2] respectively. The most interesting aspect about the data of both arteries of the healthy subjects, is that all parameters are higher for the abdominal aorta than the CCA. Therefore, it seems that the vasomotor response of the abdominal aorta is larger and has a delay with regard to the CCA. In addition, the duration of the response is longer in the abdominal aorta compared to the CCA. The mechanism causing these different responses, and stronger sympathetic stimulation, of the abdominal aorta and CCA remain unknown. A possible explanation for this might be the position of the aorta further from the hand in cold water, the fact that the diameter of the aorta is larger than the CCA, or the higher amount of SMCs. However, these are all speculations, more research is needed to draw conclusions from this.

3.4.3 Healthy subjects vs AAA patients

The difference between the vasomotor response induced by the VRT in healthy subjects and AAA patients was examined. Overall, the AAA patients responded to the VRT with a vasoconstriction and no response more often than the healthy young subjects. Furthermore, the AUC, diameter change (%), slope, AUC of response, effective diameter change and impact factor were significantly lower for the abdominal aorta of the AAA patients than for the abdominal aorta of the healthy subjects. For the CCA the mean baseline diameter, max peak, and time to peak were significantly higher in the AAA patients than in the healthy subjects. These results suggest that the vasomotor response of the AAA patients differs from the healthy young subjects, for both the CCA and abdominal aorta. In addition, the correlation between the CCA and abdominal aorta improved when adding the AAA patients, which might suggest that the CCA and abdominal aorta both changed in the presence of an AAA. This means that the endothelial function of both arteries is affected by the presence of an AAA, and the likelihood of predictive value for cardiovascular events therefore increases. The next step is studying the vasomotor response in combination with the cardiovascular outcome after a 2 year follow-up period, which will be done in the 1-2-3 Trial. However, since the CCA and abdominal aorta do have a slightly lower correlation than expected (r=0.40 for the diameter change when taking both groups together), it remains unknown whether the response of the CCA could be translated to the response of the abdominal aorta.

It was hypothesized that AAA patients showed an attenuated vasodilation or even vasoconstriction of the CCA and the abdominal aorta when performing the VRT. The results of this study demonstrate that the diameter change is indeed significantly lower for the abdominal aorta of the AAA patients compared with the young healthy subjects, -2.31% [-3.75,-0.73] vs 4.91% [2.50,8.23] respectively. The diameter change was lower for the CCA of AAA patients compared to healthy CCA, 1.36% [-3.01,2.16] vs 3.46% [-1.78,5.67], but not significant. It is striking that no significant difference was noted in the CCA of the AAA patients. This outcome is contrary to that of Makita et al. [81] who found an excessively decreased luminal distensibility of the CA in AAA patients, compared to healthy control patients. However, our group of AAA patients was incomplete and therefore quite small. Since the diameter change was almost significantly lower for the AAA patients (p=0.054), it is expected that a bigger sample size would show a significant difference.

The median of the AUC, max diameter change, AUC of response, effective diameter change and impact factor are all negative in the abdominal aorta of the AAA patients, implying vasoconstriction. This supports the hypothesis that the presence of an AAA attenuates or even reverses the vasomotor response induced by the VRT. Hence, it showed that the endothelial function of the abdominal aorta is affected by the presence of an AAA, which might help in predicting future cardiovascular events. The AUC, diameter change (%), slope, AUC of response, effective diameter change and impact factor were significantly lower for the abdominal aorta of the AAA patients than for the abdominal aorta of the healthy subjects. These results show that not only the diameter change is affected by the presence of an AAA in the abdominal aorta. It is striking that the time to peak and duration of response do not differ for both groups, while the slope, AUC of response and impact factor take time into account. This suggests that the diameter contributes to the signal to such extent that all parameters that take diameter into account are significantly different for both groups.

Furthermore, only the mean baseline diameter, max peak, and time to peak were significantly different for the CCA in both groups. These parameters were higher for the AAA patients than for the healthy subjects. These results are not surprising, the mean baseline diameter and max peak are related, and since the AAA patients suffer from a systemic dilative disease not only the aorta is affected. However, the vasomotor response was expected to be attenuated or reversed in the CCA of the AAA patients, just like the vasomotor response of the abdominal aorta, which could not directly be seen in the results. Most parameters representing the response of the CCA are lower in the AAA patients, except for the time to peak, but do not differ significantly. The time to peak is significantly higher in the CCA of the AAA patients than in the healthy subjects.

In addition, the time to peak is equal in both groups for the abdominal aorta. The healthy subjects show an increased time to peak in the abdominal aorta compared with the CCA, whereas the AAA patients have an equal time to peak for the CCA and abdominal aorta. This suggests that the time to peak is delayed for the CCA in the AAA patients.

Since there are more parameters significantly different for the abdominal aorta data of both groups than the CCA parameters, it is expected that the presence of an AAA alters the response of the abdominal aorta more than the response of the CCA. In addition, the abdominal aorta shows more vasoconstriction and no response than the CCA in the AAA patients. However, the AAA group shows considerable more vasoconstriction and no response compared to the healthy young group.

3.4.4 Limitations

The reader should bear in mind that the study is based on interim results of an ongoing study (31 out of 60 included). As stated in the introduction of this thesis, ageing could affect the response on sympathetic stimulation. Van Mil et al. [13] showed a diameter change of 1.8 ± 2.6% in older patients (61 ± 8 years old), and a diameter change of 4.1 ± 3.7% in young patients (24 ± 3 years old). The ages of the present study (69 [68,77] and 24 [21,26] years old), and the diameter changes (1.36% [-3.01,2.16], 3.46% [-1.78,5.67]) were comparable to the results van Mil et al. [13] showed. Therefore, it is important to compare the results of the AAA patients not only to the young healthy subjects, but also age matched subjects in order to examine the effect of the presence of an AAA independent of age. Measuring age matched subjects, together with increasing the sample size of the AAA patient group, is the next step of the RESPONSE study.

Furthermore, the algorithm from the BloodFlow Software is a black box and many details of the analysis are, unfortunately, unknown. For example, there was a discrepancy in the frame rate of the AVI files and the data gained from the BloodFlow Software, which might cause an error in time to peak and duration of response and makes the ability to filter the data for heart rate and respiratory rate impossible. Further data collection into this topic is required to determine exactly how this discrepancy affects the outcome of the VRT. The frame rate issue will be examined into further detail in chapter 4. In addition, the main challenge with B-mode US is to identify clear vascular boundaries. Imaging of the artery in the longitudinal plane allows for precise diameter measurement (≤ 0.05 mm) by this automated edge-detection software [70].

The measurements were mostly performed by trained researchers and a few by sonographers of the vascular center, meaning that not all measurements were performed by the same sonographers. The sonographers had to prevent probe movement during the measurement. In addition, at the different locations, different probes and US equipment were used. However, the potential errors due to these limitations cannot be the explanation of the unexpected findings of this study, since the data is both manually and automatically filtered for measurement artefacts. In addition, the CCA is measured with a linear probe and the abdominal aorta is measured with a curved probe. This probe has a greater ROI, a frequency matching the deeper anatomy, and is therefore more versatile and can create more optimal images of the abdominal aorta, especially in overweight subjects. Since both probes have different specifications, the influence of this on the diameter measurements will be studied into more depth in chapter 5.

The data of two patients could not be used, due to extensive thrombotic plaque formation, disturbing the wall tracking algorithm, and low quality US measurement. In this study, the AAA measurements were performed right above the bifurcation. This led to variation in the location of the US measurements, because some aneurysms start right above the bifurcation and others more cranial. Besides these differences in location, differences could be present due to the fact that AAAs could be saccular and fusiform, could have extensive thrombotic plaque formation, or intestinal gas could affect the quality of the US measurement. Since the diameter analysis is done on the AAA vessel wall itself, good quality measurements are fundamental. However, more data on patients with extensive thrombus in the AAA would give more insight into the vasomotor response of the lumen. 69% of the AAA patients have intraluminal thrombus [82]. If it appears that the responses of the lumen are comparable to the responses of the vessel wall, the VRT can also be used for patients with extensive thrombus.

The classification threshold is set at (-)1.5% diameter change, based on earlier experiences in the RadboudUMC with the VRT on the CCA in young healthy patients and PAD patients. There is no gold standard for this classification, and the limitations of the traditional method are known (e.g. insecurity caused by the visual classification of the curve). To date, there has been no experimental evidence whether the used threshold is applicable for the abdominal aorta, but since it is the only known value it has been decided to use this value. Since the aim was to use the VRT as a predictive method for cardiovascular events in AAA patients with regard to the classification, the final threshold should be determined based on the cardiovascular outcome.

3.4.5 Clinical relevance

These results show that a more objective and mainly automated examination of the VRT can be done using the refined method, which gives confidence for the use of the refined method and its new parameters. Furthermore, it gives insight into the vasomotor response of the abdominal aorta and the CCA of AAA patients, which could be used for the development of a non-invasive method for the prediction of cardiovascular events in AAA patients. The initial idea was to monitor the CCA during the VRT, since this is an easy to measure, superficial artery. However, this study suggests that the vasomotor response of the abdominal aorta is affected by the presence of an AAA more than the response of the CCA. Therefore, it is thought that the VRT of the abdominal aorta would improve the reliability of making a distinction between healthy subjects and AAA patients. However, more data of AAA patients and healthy older subjects is needed to make a reliable decision on the measurement method. Furthermore, it is recommended to use a statistic test that pairs the arteries, to study the ability to make a distinction between the healthy subjects and the AAA patients. With this information, it can be determined which artery is preferred to perform the VRT measurements on. In addition, the CV outcome is needed to make a proper decision.

Another clinical relevant outcome that is shown by this study, is the fact that the abdominal aorta of one of the AAA patients shows a 7.5% diameter change in response to the VRT. It is known that some EVAR patients have an unexplainable endoleak. When an EVAR is placed, anchoring segments need to provide sufficient sealing and fixation in the aortic neck. Therefore, most devices rely on some degree of oversizing, varying from 10% to 25% [17]. In one AAA patient the vasomotor response of the aortic neck was measured, as a feasibility test of the analysis software. The aortic neck showed an increased and reversed response with regard to the AAA (3.5% and -5.9% respectively). For patients with a higher response of the AAA neck, e.g. the AAA patient with the 7.5% dilation of the AAA, it is expected that the diameter change possibly rises above the degree of oversizing. This might explain some types of endoleak, when sympathetic stimulation occurs in a patient treated with an EVAR. On the other hand, the CCA of this patient showed a response of 3.1% which is slightly lower than the aorta of this patient. The aortic neck therefore seems to have a better correlation with the CCA than the AAA. This contradicts the improved correlation when taking both groups into account. Hence, more data on the aortic neck of AAA patients is needed in order to study the correlation between the CCA and the abdominal aorta and the possible effects on EVAR treatment.

3.5 Conclusion

In conclusion, it is suggested that the determined parameters are equal for both the traditional and refined method, which gives confidence for the use of the refined method with regard to the examination of the vasomotor response. The correlation of the vasomotor response between the abdominal aorta and the CCA is lower than expected, and might suggest that the vasomotor response of the abdominal aorta deviates from the response of the CCA in young healthy subjects. However, when taking both groups into account the correlation improves. The correlation is expected to further improve when increasing the sample size, which is therefore recommended. Furthermore, the vasomotor response is altered in the presence of an AAA. However, since the CCA and abdominal aorta do not show the same response, it remains unknown whether the results of the VRT on the CCA could be translated to the response of the abdominal aorta on the VRT.

CHAPTER

4 Spectral analysis

4.1 Introduction

Sampling is the act of measuring a continuous time signal $x(t)$ at discrete time instances. The time between two consecutive instances, T_s, is called the sampling period or sampling interval. The number of samples per unit time is the sampling frequency, f_s, giving Equation 4.1.

$$f_s = \frac{1}{T_s} \tag{4.1}$$

The Fourier transform decomposes a function of time into its constituent frequencies. Spectral analysis deals with the problem of determining the distribution of power over frequency of a finite record of time series [83]. The classical (or non-parametric) methods of spectral analysis consider a band-pass filter with a narrow bandwidth, which is swept through the frequency band of interest. The filter output power is divided by the filter bandwidth in order to use as a measure of the spectral content of the input to the filter. The second approach to spectral estimation (parametric) is to postulate a model for the data.

Energy spectral density describes the distribution of the energy of a signal or a time series with frequency. The energy spectral density is most convenient for pulse-like signals having a finite total energy. However, the most used version of the Fourier transform is the periodogram, $P_{xx}(f)$. The periodogram incorporates the power of the signal, which is the average of the squared magnitude of the signal (x(t)) over the given epoch (T) (Equation 4.2).

$$power(x) := \frac{1}{T}\int_{-T/2}^{T/2} |x(t)|^2 dt \tag{4.2}$$

The periodogram $P_{xx}(f)$ is then defined as:

$$P_{xx}(f) = \alpha|\hat{x}(f)|^2 \tag{4.3}$$

where the scaling factor α is chosen such that the integral over all frequencies equals the power of the signal (Equation 4.4), and x(f) is the signal x in the frequency domain.

$$power(x) = \int_{-\infty}^{\infty} P_{xx}(f) df \tag{4.4}$$

The area under the curve (AUC) of the periodogram is the power of the signal. Therefore, the periodogram is often called the power spectral density (PSD). PSD is more commonly utilized, which applies to signals existing over all time or a large enough time period in relation to the duration of a measurement.

The BloodFlow Software (Version 5.2; National Instruments LabVIEW, Austin, TX, USA) used for the determination of the diameters of the CCA and the abdominal aorta gives a discrete time signal. The algorithm of the diameter determination by the software is explained in section 3.2.4 and 5.1.

Since the diameter of an artery is measured, it is expected that the heart rate (HR) is present in the signal. The measurement of the abdominal aorta could also be affected by respiration, hence it is expected that both the HR and respiratory rate (RR) are present in the signal of the abdominal aorta. The use of a periodogram or PSD can play an essential role in analysing the measured diameters of the arteries with regard to the HR and RR in particular. A periodogram visualizes the frequencies contributing significantly to the signal. If the HR and RR contribute significantly

to the signal, filtering of these frequencies is crucial to ensure measurement of the response during the VRT regardless of the influence of HR and RR on the signal.

The periodogram function uses the sample frequency (Equation 4.1), and thus the sampling period. When studying the time and diameter data set into further detail, a discrepancy in sample frequency is noticeable. It is seen that the sample frequency from the software differs from the frame rate of the US video, in particular when the velocity is measured in addition to the diameter. Furthermore, the duration of the video in the software differs from the actual duration of the video. What is known about the discrepancy comes from observations, using data from the RESPONSE study. It is suggested that it is caused by the used BloodFlow software. The discrepancy in sample frequency and its consequences are an important, but understudied, cause for concern. The goal of this section is to investigate the influence of the discrepancies in frame rate on the diameter determination.

4.2 Method

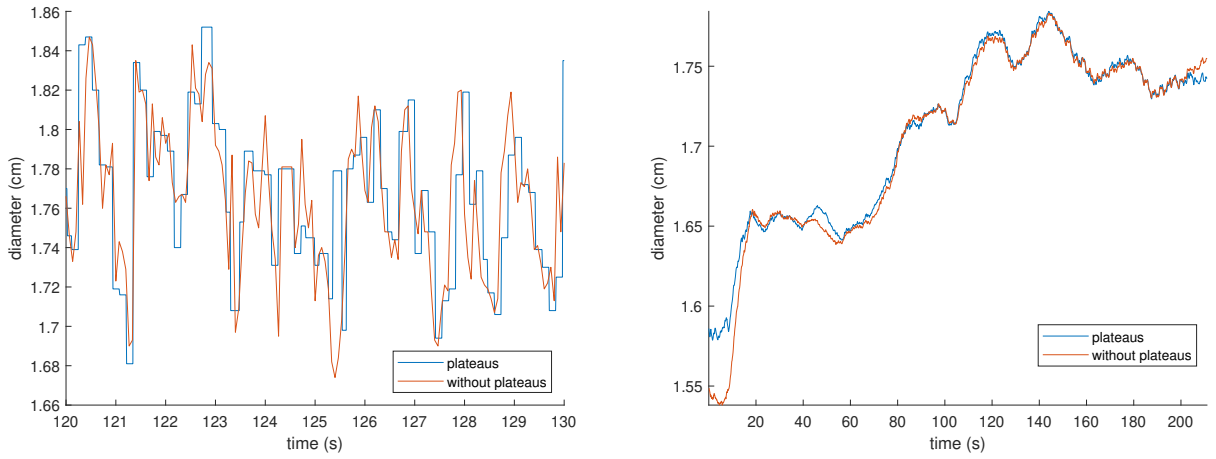
The diameter and velocity of the abdominal aorta of three young healthy subjects was measured during the VRT, using the protocol described in section 3.2. The measured data was saved as an Audio Video Interleave (AVI) file, and this AVI file was loaded into the BloodFlow software. The settings of the software were set at the Advanced Edge analysis method, using a line thickness of 2. The analysis was once done for both the diameter and velocity and once for only the diameter.

For both data sets the diameter was filtered in Matlab (R2018b; The Mathworks, Inc., Natick, MA, USA) using a threshold of 2.5 * Median Absolute Deviation (MAD) for the baseline and CPT separately. Then the missing data was filled using linear interpolation and a moving mean of the data was calculated using a 10 second time window.

4.3 Results

When studying one subject, the sample frequency of the US video differs from the sample frequency of the data set. Moreover, not only the sample frequency changes, but also the duration of the video increases when using the software. Furthermore, the sampling period within the data set happens to differ for several subjects as well. Besides, it is noticed that there were 'plateaus' visible in the diameter of the data set when both the diameter and velocity were analyzed using the software. At these plateaus the diameter is constant for a few number of samples, while the velocity had unique values. Figure 4.1 shows the diameter of the abdominal aorta of a healthy subject during the VRT, with and without the plateaus caused by the software, with Subfigure 4.1a showing the raw data for one subject during 10 seconds of the VRT. Subfigure 4.1b shows the filtered data during the VRT for both the method with and without the plateaus.

The maximum diameter change (%) measured using the VRT is 9.7% and 10.8% for the 'plateau' data and the data without plateaus respectively in this healthy subject. For two other subjects the same was done, which showed diameter changes of 3.8% & 3.3%, and 17.6% & 18.4%.



(a) Raw data during 10 seconds of the VRT

(b) Filtered data during the VRT

Figure 4.1: Diameter of the abdominal aorta of a healthy subject during the VRT, with and without the plateaus caused by the software.

4.4 Discussion

The results of maximum diameter change (%) induced by the VRT indicate that the plateaus do not affect the outcome to a great extent.

Subfigure 4.1b shows the filtered data during the VRT for both the method with and without the plateaus, which shows two approximately equal signals.

Due to the discrepancies in frame rate and duration of the video, a periodogram of this data is not applicable, since the periodogram is made using one frame rate. This frame rate is not consistent during the measurement. Therefore, there could not be corrected for the HR and RR by filtering these frequencies out of the signal. However, since the signal is smoothed using a moving mean with a ten second time window, the HR and RR will be averaged as well and the extent to which the signal is affected by the HR and RR is minimized.

In order to explain the discrepancies of the frame rate and duration of the video further knowledge about the software was needed, hence we contacted the software developer. He explained that the software derives its timing from the US video frame rate value, where it reads the fixed frame rate value from the header of the US video. However, the frame rate of the video is not always equal to the frame rate in the software, and therefore an error in the timing can occur. The error might occur due to missing/skipping a frame, which could happen in both the software and/or the encoding of the US video. The error in timing slightly affects the interpretation of the measured response during the VRT, e.g. the time of maximum diameter change might be misinterpreted by a few seconds.

4.5 Conclusion

In conclusion, the data set could not be filtered to eliminate the influence of HR and RR, and the software contains some minor errors which slightly affect the outcome. Since the filtering is an extra processing step next to the regular smoothing of the signal this barely affects the outcome and changes due to the minor errors are considered negligible. However, after improvements of the software to eliminate the errors, it is recommended to use filtering for the HR and RR.

CHAPTER

5 Abdominal probe test

Acoustic wave propagation through a heterogeneous medium is associated with energy loss. This progressive weakening of the acoustic wave is called attenuation [84]. Major causes of this attenuation are scattering and absorption. Due to this loss of energy, the distance travelled in the medium as well as emitted central frequency alters. This phenomenon is described in Equation 5.1.

$$A(z, f_0) = A_0 e^{-\alpha f_0 z} \tag{5.1}$$

In this equation, A is the amplitude of the propagating wave, z and f_0 are the depth travelled and emitted central frequency, A_0 is the initial intensity of the wave at emission, and α represents the attenuation coefficient [85]. The initial amplitude decreases exponential as a wave propagates further into the tissue. The attenuation is frequency dependent, where a higher frequency is subject to more attenuation than the lower frequency components [85]. To avoid attenuation at the desired distance travelled in the medium, a specific range of frequencies can be used. Therefore, deeper anatomy, like the abdominal aorta, uses frequencies in the range of 1 to 3 MHz and superficial anatomy, such as the carotid artery, uses frequencies in the range of 5 to 10 MHz.

The linear array probe is designed for superficial imaging. This probe has this higher frequency range and therefore produces better axial resolution and less penetration. The piezoelectric crystals are aligned in a linear way and produce sound waves in a straight line, with a constant lateral resolution [86]. In a convex, curved, probe the crystals are embedded in a curved array and the frequency range is lower. The ultrasound (US) beams in a curved probe fan out more with distance, which causes the lateral resolution to reduce in deeper tissue [84].

In the RESPONSE study (chapter 3) it was decided to use a curved probe for the aortic measurements. However, the carotid artery was measured using the linear probe because of the superficial location. In order to determine a proper correlation between the carotid artery and the abdominal aorta, the differences between the curved and linear probe when using it for the measurement of vasoreactivity during the vasomotor reactivity test (VRT) need to be studied. Furthermore, the feasibility of the analysis software for determining the diameter has to be tested for the curved probe, with and without the VRT.

5.1 Abdominal probe test: without VRT

5.1.1 Objective

The goal of this part is to study the differences between the curved and linear probe when measuring the diameter of the abdominal aorta. In addition, it is aimed to determine if the analysis software is able to determine the diameter of the abdominal aorta when using the curved probe.

5.1.2 Method

The study population consisted of 4 healthy young females. All volunteers had to be between 18 and 40 years of age and the only exclusion criterion for this feasibility study was a BMI>30 kg/m^2. This study is a feasibility study, and therefore no permission is needed from the ethics committee. Besides, a formal sample size calculation is not required. There is aimed to include 4 participants, because it was felt this would be a large enough sample to test the ability to measure the diameter of the abdominal aorta using both probes.

For this feasibility study two consecutive US measurements were performed on the abdominal aorta of all subjects in the longitudinal plane. The first measurement was done using a linear probe (L9-3, Philips, Amsterdam, the Netherlands) and for the second measurement a curved probe (C5-1, Philips, Amsterdam, the Netherlands) was used. After the probe was placed right above the aortic bifurcation and finding an optimal image (where the vessel wall of the abdominal aorta are clearly visible), the probe had to be held stable and US parameters were set to improve the contrast, and thereby the visibility of the lumen-arterial wall interface. Two, 2-minute measurement sessions were performed for each subject. Only subject 1 had a 2:24 min measurement for the curved probe. Both measurements were performed by the same researcher.

The measured data was saved as an Audio Video Interleave (AVI) file. The AVI files were loaded into the BloodFlow Software. The analysis of this data was executed as described in section 3.2.

This group of four subjects is too small to perform statistical tests. Therefore, an acceptable error was stated with regard to the physiology. Since the expected difference in diameter of the abdominal aorta induced by the VRT lies in the range of 1.51 ± 0.56 mm for nonsmokers and 0.54 ± 0.71 mm for smokers [15] and the best obtainable accuracy of the curved probe is 0.31 mm (with a wavelength of 5 MHz), the acceptable error will be ≤ 0.35 mm. This is also in agreement with the quality of the measurements, since the expected diameter change is bigger than this error.

In order to investigate whether the error originates from the probe and not the software, we performed some manual measurements. Three random diameters of the vessel lumen per frame were manually measured per performed measurement, using IC Measure (version 2.0.0.161, The Imaging Source Europe GmbH, Bremen, Germany). This allows manual on-screen image measurement, and the distances were calibrated using the scale of the US video. The mean of three diameters were calculated for every measurement (both curved and linear for each subject), which was compared to the mean diameter given by the software.

5.1.3 Results

Four subjects were included (all female) with a median age of 23 [23,23.5] years old and a median BMI of 23.9 [21.8,25.4] kg/m^2. The mean diameter was calculated for all measurements, and the percentage of removed outliers has a mean of 7.8 ± 5.4% of the total dataset.

Figure 5.1 shows the diameter of the abdominal aorta over time for the curved (red line) and linear probe (blue line) with standard deviation for subject 1. It shows that the linear probe measured a higher diameter of the abdominal aorta than the curved probe. The mean diameter of the linear probe in this subject is 13.7 ± 0.1 mm and the mean diameter of the curved probe is 12.0 ± 0.1 mm. The mean diameters of all subjects are shown in Table 5.1. This table also shows the diameter differences between the measurements of the linear and curved probe, which has a mean of 1.4 ± 0.3 mm. All of these diameter differences are ≥ 0.35 mm.

Table 5.1: Mean diameter values of the abdominal aorta measured by the linear and curved probes

Subject	Diameter, Linear (mm ± SD)	Diameter, Curved (mm ± SD)	Diameter difference (mm, linear-curved)
1	13.7 ± 0.1	12.0 ± 0.1	1.8
2	13.0 ± 0.1	11.8 ± 0.2	1.2
3	15.0 ± 0.2	13.8 ± 0.1	1.1
4	13.0 ± 0.1	11.3 ± 0.1	1.7

Table 5.2 shows the manual measured diameter, the diameter determined by the software and the difference between these two. When measuring manually the diameter measured by the curved probe is not equal to the linear probe, just like the diameters measured by the software. The diameter difference between the manual measurements of the linear and curved probe has a mean of 0.8 ± 0.5 mm. The absolute difference between manual and software has a mean of 0.4 ± 0.4 mm.

5.1.4 Discussion

The goal of this feasibility study was to determine if the analysis software was able to determine the diameter of the abdominal aorta when using the curved probe. In addition, there was aimed to study the differences between the curved and linear probe when measuring the diameter of the abdominal aorta. The diameter of the abdominal aorta measured with the linear probe was higher than using the curved probe for all subjects. It can be concluded that the measured difference between the curved and linear probe is not acceptable with regard to the physiology. However, the difference between the linear and curved probe was not constant. This indicates that it is not only the accuracy of the

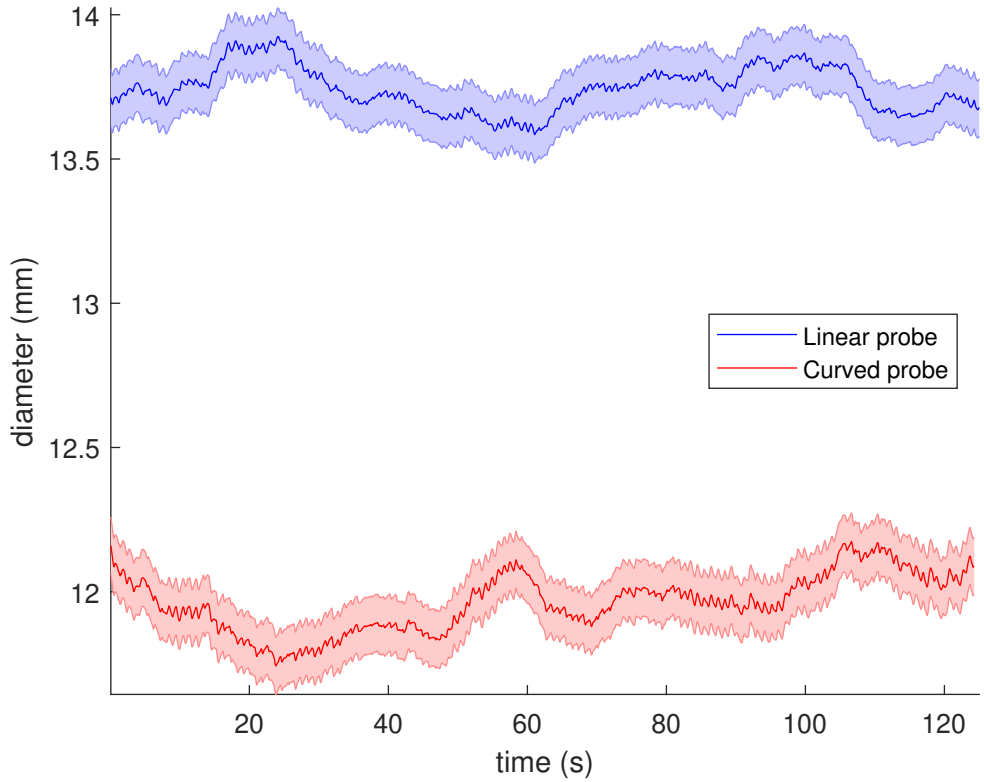


Figure 5.1: Diameter of the abdominal aorta over time for the curved and linear probe with standard deviation in subject 1

Table 5.2: Manual measured diameters in mm, where C stands for Curved and L stands for Linear

Subject	Probe	Manual	Software	Absolute difference manual and software
1	C	13.1	12.0	1.1
	L	13.8	13.7	0.1
2	C	12.7	11.8	0.9
	L	12.6	13.0	0.4
3	C	13.9	13.8	0.1
	L	15.0	15.0	0.0
4	C	11.6	11.3	0.3
	L	12.9	13.0	0.1

probe that causes the error, but possibly also probe manipulation, investigator dependency and the use of the linear probe on a deeper located vessel [87].

Furthermore, when measuring manually the diameter measured by the curved probe is not equal to the linear probe, just like the diameters measured by the software. However, the diameter difference determined by the software and manual (0.4 ± 0.4 mm) is about 3.5 times smaller than the difference between the curved and linear probe using the software (1.4 ± 0.3 mm). This indicates that the discrepancy between these two probes cannot be explained by the use of the software, since it does not affect the diameter to such an extent that it can explain the difference between the measured diameters of the curved and linear probe. Therefore, it can be concluded that the software can be used for measuring the diameter of the abdominal aorta using the curved probe.

This result suggests that there is a discrepancy between the curved and the linear probe. This can be explained by a lot of factors, e.g. probe manipulation, depth of the artery in combination with the used frequency and alignment of the piezoelectric crystals. In order to say how this affects the VRT measurements it has to be investigated if the measured response and thus diameter change is similar for both the curved and linear probe.

When using ultrasound there is a limited resolution achievable, which causes an error in diameter measurement. The best obtainable accuracy is of the order of the smallest wavelength of the transmitted ultrasound [88]. The wavelengths of ultrasound frequencies of 5 MHz (curved probe) and 12 MHz (linear probe) are respectively 0.31 mm

and 0.13 mm, representing the best obtainable accuracy. Considering this accuracy the error, especially on small vessels, can be very high. Deviations smaller than the best obtainable accuracy can therefore not be explained in our data set. Scorza et al. [87] studied the influence of probe placement on quality of the US measurement by using a linear and a curved probe, which showed that the measurements strongly depend on settings as well on probes and parameters. Furthermore, the relative uncertainty on spatial resolution can range from 10 to more than 30 percent, due to probe manipulation by the investigator. In convex array probe higher uncertainties in spatial resolution are related mainly to deep targets.

The test-retest reliability of US measurements on the rectus femoris thickness showed a high intraclass correlation for both the curved and linear probe (0.87-0.97) [89, 90]. Similar to the results in this study, Nijholt et al. [90] measured a greater thickness of the rectus femoris with the linear probe compared to the curved probe. These results suggest that both probes could be used on different measurement moments without affecting the results.

However, in a clinical setting the diameter of the abdominal aorta is measured in the transversal plane. In addition, the curved probe is always used for these measurements, which means that the linear probe does not have to be compared to the curved probe in a clinical setting.

5.1.5 Conclusion

It can be concluded that the error between the curved and linear probe is not acceptable with regard to the physiology for measuring the diameter of the abdominal aorta in the longitudinal plane. There is a discrepancy between the curved and linear probe, when measuring the abdominal aorta diameter. The diameter of the abdominal aorta measured with the linear probe is higher than using the curved probe for all subjects, although not with a constant error. Furthermore, it can be concluded that the analysis software is able to determine the diameter of the abdominal aorta while using the curved probe, since the difference between the software and the manually determined diameter is three times smaller than the difference between the curved and linear probe. Further research is needed to investigate the effect of the discrepancy between the curved and linear probe on the measured diameter change during the VRT, because of the interest in the relative, and not the absolute, diameter change.

5.2 Abdominal probe test: during VRT

5.2.1 Objective

The goal of this test is to investigate the effect of the discrepancy between the curved and linear probe on the measured diameter change during the VRT. In order to say how this affects our VRT measurements it has to be investigated if the measured response and thus diameter change is similar for both the curved and linear probe.

5.2.2 Method

The study population consisted of 3 healthy young participants. All volunteers had to be between 18 and 40 years of age. Exclusion criteria for this feasibility study were:

- Increased risk for coronary spasms (score Rose-questionnaire ≥ 2; this questionnaire can be found in Appendix 8.1)
- BMI>30 kg/m^2
- Presence of Raynoud's phenomenon, Marfan syndrome, chronic pain syndrome at upper extremity(s), presence of an AV fistula or shunt, open wounds to the upper extremity(s), and/or scleroderma associated with placing the hand in ice water
- Recent (<3 months) presence of angina pectoris, myocardial infarction, cerebral infarction, and/or heart failure, or PAD treatment

This study is a small pilot study, and therefore no permission is needed from the ethics committee. Since this is a feasibility study a formal sample size calculation is not required. There was aimed to include 3 participants, because it was felt this would be a large enough sample to test the ability to measure the diameter change of the abdominal aorta using both probes.

Two ultrasound measurements were performed on the abdominal aorta of all subjects during the CPT on two consecutive days. The first day, the measurement was performed using a linear probe (L9-3, Philips, Amsterdam, the

Netherlands) and the next day, the measurement was done using a curved probe (C5-1, Philips, Amsterdam, the Netherlands). This is the only difference between the performed measurements. The subjects were given instructions to keep their food intake with a high vitamin C content, caffeine and alcohol intake, and physical activity equal 24 hours prior to the measurements. The measurements were also performed at the same time of the day.

After the subject had been resting for 10 minutes in supine position, the blood pressure was measured twice (T=0). The probe was placed right above the aortic bifurcation and an optimal image had to be found, where the vessel wall of the abdominal aorta is clearly visible in the longitudinal plane. The probe had to be held stable and US parameters were set to improve the visibility of the lumen-arterial wall interface. When the optimal settings are found, the VRT can start with a 30 second baseline US measurement on the abdominal aorta. After this baseline measurement the hand of the subject was positioned into the ice water for a duration of three minutes. The temperature of the ice water was 4 degrees Celcius for all subjects. The blood pressure is measured after each minute of the CPT (T=1, 2 and 3), using the Microlife WatchBP Home sphygmomanometer (Microlife corporation, Taipei, Taiwan).

The measured data was pre-processed in the same way as described in section 2.2.2. From the described parameters in this section, only the maximum diameter change (%) was calculated.

This group of three subjects is too small to perform statistical tests. Therefore, an acceptable error was stated for the difference between the curved and linear probe with regard to the physiology. Since the expected diameter change of the abdominal aorta measured during the VRT lies in the range of 10.7% ± 4.0% for nonsmokers and 3.8% ± 5.0% for smokers [15], the acceptable error between the probes will be 3.0%.

5.2.3 Results

One male and two female subjects were included in this study, with a median age of 27 [26,31] years old, and a median BMI of 25.9 [25.1,26.4] kg/m^2. The maximum diameter change could be determined for all subjects, and the percentage of removed outliers has a mean of 14.7 ± 10.5% of the total data set. Every subject showed a response on the sympathetic stimulation by an increase of systolic and diastolic blood pressure.

Table 5.3 shows the maximum diameter change for all subjects for both the curved and linear probe, with a median of -3.63% [-6.40,2.71]. The classification of the vasomotor response is different in two out of three subjects. The curved probe measurement of subject 1 shows vasoconstriction, whereas the linear probe measurement of this subject shows vasodilation. In subject 2 the curved probe measurement shows vasodilation, whereas the linear probe measurement shows vasoconstriction. Subject 3 shows vasoconstriction twice during the VRT. The absolute differences in maximum diameter change are respectively 9.22%, 7.95% and 5.33%, which means that all errors are ≥3%, and therefore not acceptable with regard to the physiology.

Table 5.3: Maximum diameter change (%) of the abdominal aorta measured by the linear and curved probes

Subject	Linear probe	Curved probe
1	4.44	-4.78
2	-2.49	5.46
3	-12.27	-6.94

Figure 5.2 shows the diameter of the abdominal aorta over time for the curved and linear probe with standard deviation during the VRT in subject 1. The linear probe measures a greater diameter than the curved probe. Furthermore, this figure shows that the maximum diameter change is smaller than the difference between the two probes, which is the case for all three subjects.

5.2.4 Discussion

For this test the goal was to investigate the effect of the discrepancy between the curved and linear probe on the measured diameter change during the VRT. Two of the subjects responded differently on the two measurements, i.e. vasodilation and vasoconstriction. The other subject responded with vasoconstriction twice. None of the subjects showed an acceptable difference between the two measurements. Therefore, it cannot be concluded that the discrepancy between the curved and linear probe does not affect the measured diameter change during the VRT. On top of that, even the response classification, i.e. vasodilation and vasoconstriction, changed as a result of the diameter change. The sample size of this pilot was very small, because it was thought it would have been enough to show a general response between the curved and linear probe. Subject 3 had low quality US measurements, but since the sample size was this small there was decided to still take the data into account. For a larger sample size ethical approval would be necessary.

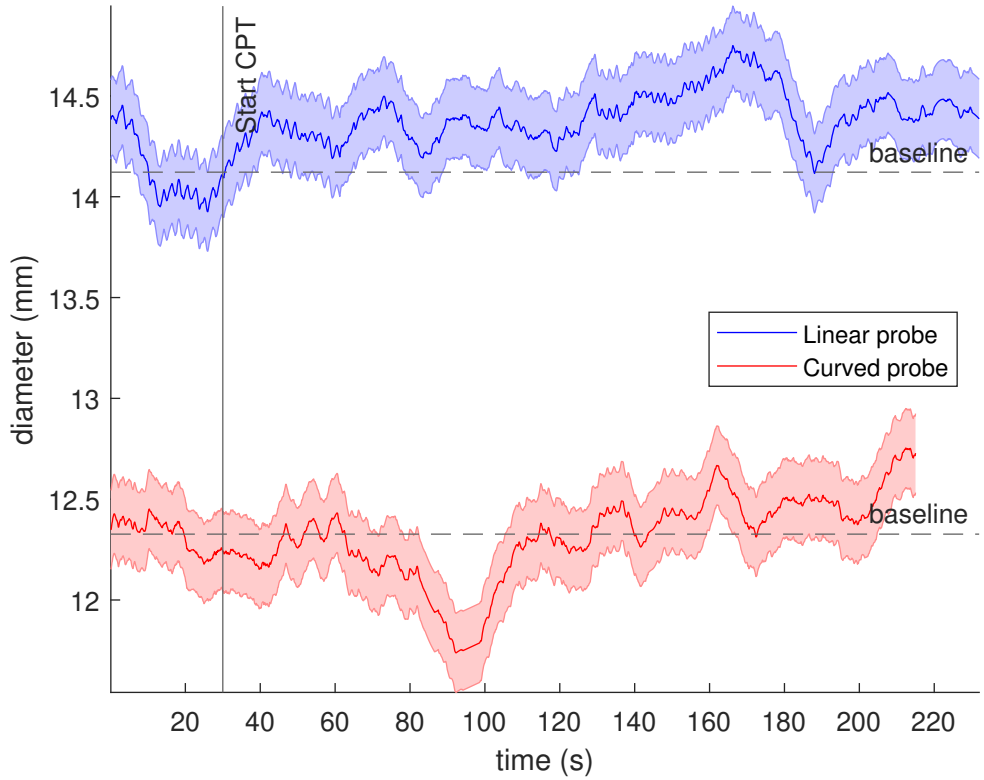


Figure 5.2: Diameter of the abdominal aorta over time for the curved and linear probe with standard deviation during the VRT in subject 1. The red line shows the curved probe measurement, and the blue line shows the linear probe measurement. The vertical black line indicates the start of the CPT, and the horizontal dashed lines show the mean baseline diameter of both measurements.

Since the sample size was this small and there is a large amount of factors that could possibly influence the outcome, it remains unknown if the different probes cause the deviating outcome.

The subjects were given instructions to keep their food intake with a high vitamin C content, caffeine and alcohol intake, and physical activity equal 24 hours prior to the measurements. According to a physiological guideline these factors should be avoided when performing the VRT [70], since it is known that these factors can have an effect on endothelial function and therefore influence the response on the VRT [71–73]. Since they were only told to equal these factors over the two days instead of abstinence, this could have affected the outcome of the VRT.

For the carotid artery reactivity test the test-retest reliability is investigated by a co-investigator. This showed errors of 2.60%, 2.10%, 2.77% and 0.11%. Therefore, it is assumed that deviations of this degree are acceptable with regard to the physiology. An error of 2.8% can occur based on the day of measurement, which can possibly explain part of the outcome of this test [13].

5.2.5 Conclusion

It remains unclear to what degree the discrepancy between the curved and linear probe affects the measured diameter change during the VRT. Hence, the research question could not be answered. With this small sample size and a large amount of factors that could possibly influence the outcome, it remains unknown if the different probes cause the deviating outcome.

CHAPTER

6 Future perspective

A refined method was developed for the examination of the vasomotor response during the VRT. Despite the promising results of this method, questions about the exact use of the determined parameters remain. Further studies, which take these parameters into account, will need to be undertaken. The correlation of the vasomotor response of the abdominal aorta and CCA is lower than expected, and might suggest that the vasomotor response of the abdominal aorta deviates from the response of the CCA. Besides, the vasomotor response is altered in the presence of an AAA, in both the abdominal aorta and the CCA. This information is of major importance for the development of a non-invasive method to predict cardiovascular events in AAA patients, as this phenomenon is related to an increased incidence in patients with PAD. What remains unknown, and is thus recommended for future research, is the vasomotor response in elderly subjects. This will be done in the continuation of the RESPONSE study. Additionally, another project will investigate the vasomotor response of the CCA in AAA patients in combination with the cardiovascular outcome in a two year follow-up.

Based on observations made during the RESPONSE study, experiments on the spectral analysis and probe choice were performed. Results of the spectral analysis were able to clarify the discrepancy in frame rate and duration of the video, and showed that these were considered negligible. However, after improvements of the software, filtering for the HR and RR is recommended. The influence of the curved or linear probe on the measured diameter change during the VRT remains unknown. To develop a full picture of the influence of probe choice on the measured vasomotor response during the VRT additional studies will be needed that use a greater sample size.

During this project, a much debated question was the measurement and analysis method of the AAA. In this study all measurements were performed right above the bifurcation, and the analysis using the software is done on the AAA itself. However, a few AAAs could not be used in this method due to e.g. differences in anatomical location or extensive thrombotic plaque formation. The development of a universal method to measure the vasomotor response of an AAA is needed, especially if it turns out that the vasomotor response of the abdominal aorta has better prognostic value than the CCA. The results of the RESPONSE study show that the vasomotor response of the abdominal aorta is affected to a greater extent than the vasomotor response of the CCA. This information might imply that the vasomotor response of the abdominal aorta has better prognostic value, and further research into this subject is recommended.

In addition, the AAA has a heterogeneous vessel wall. Therefore, it is expected that the abdominal aorta in AAA patients does not have a circular equal response. Hence, the vasomotor response might be heterogeneous when performing the VRT. Conventional ultrasound is unable to measure the vasomotor activity in multiple directions, which yields a distorted image if it is true that the vasomotor response inside the AAA is heterogeneous [25]. Three-dimensional (3D) US provides a more accurate image plane orientation and diameter estimation than 2D US [91]. The data also provides information regarding the AAA size along its entire length, which is useful when determining vasomotor response of the abdominal aorta and the AAA in particular. Therefore, this technique could help in giving insightful information about the vasomotor activity of the abdominal aorta, and determine the universal measurement method of an AAA.

In conclusion, the VRT for the prediction of cardiovascular events in AAA patients needs to be studied into more depth. Future studies on the current topic are therefore recommended. The clinical use of the VRT could be implemented with the duplex US measurements that are performed on AAA patients who are under surveillance, together with cardiovascular risk management. Ideally, if the VRT could actually predict cardiovascular events, there can be decided to perform the VRT on an annual basis and the cardiovascular drug use could be adjusted to the outcome of the VRT. Furthermore, thoughts are that the outcome of the VRT could be used for risk assessment prior to treatment of the

AAA. The onset of cardiovascular events in AAA patients and the predictive value of the vasomotor response of the CCA measured by the VRT, is currently studied in the 1-2-3 Trial. Another project will start soon, the one-two-treat Trial, measuring the VRT on the CCA in AAA patients that are surgically treated. This will provide even more information about the use of the VRT in AAA patients. As described earlier, sympathetic stimulation in EVAR patients might explain some types of endoleak, which could be of major importance for the treatment of these patients.

CHAPTER

7 Case report: Embolization of the false lumen of an infrarenal post-dissection aneurysm using IMPEDE-FX Embolization Plugs

7.1 Abstract

Purpose: To demonstrate the value of IMPEDE-FX embolization plugs to embolize a false lumen of an infrarenal post-dissection aneurysm. **Case Report:** A 69-year-old patient was treated with a mitral valve replacement, complicated by a Crawford type A dissection. After 9 years he presented with an enlarging infrarenal post-dissection aneurysm with a maximum diameter of 81 mm and a 56 mm true lumen diameter. The entire false lumen was embolized using multiple IMPEDE-FX embolization plugs followed by a covered stent placement over the entry tear in the right iliac artery. At 8 months a CTA showed a fully thrombosed false lumen. The total AAA diameter remained stable but there was a clear remodeling of the true lumen with a diameter reduction to 52 mm. The volume of the true lumen showed a similar decrease from 102.4 cm^3 preoperatively to 87.5 cm^3 at 8 months. **Conclusion:** Embolization of a false lumen of an infrarenal post-dissection aneurysm can be performed using IMPEDE-FX embolization plugs. Confirmatory prospective trials on patients with non-thrombosed false lumina are indicated.

7.2 Introduction

Persistent false lumen filling is associated with delayed or failing remodeling of post-dissection aorta, resulting in an increased risk of aneurysm formation, rupture and death. Thrombosis of the false lumen promotes post-dissection aortic remodeling. Endovascular strategies aimed at promoting full thrombosis of the false lumen mostly focus on covering the entry-tear with a covered stent [92, 93]. In cases where covering of the entry-tear is unsuccessful, embolization of the false lumen could be considered. Several techniques for false lumen embolization have been described, such as the use of conventional coils, plugs, glue and iliac limb occluders [94, 95]. These techniques are usually not suitable for large false lumen diameters. Kolbel et al. [96] developed an extra-large vascular plug for the occlusion of a large distal false lumen in chronic aortic dissection, the Candy-Plug (Cook Medical, Bjæverskov, Denmark). However, this is not CE approved to date and remains to be a custom-made device.

The IMPEDE-FX Embolization Plug (Shape Memory Medical, Santa Clara, CA, USA) is a novel embolization device consisting of a self-expanding Shape Memory Polymer (SMP) Plug and a proximal platinum/iridium marker band [97]. SMP is a porous, biocompatible and non-inflammatory polymeric scaffold, made of polyurethane, that is able to turn back into its memorized shape. The device is supplied preloaded in an introducer with the SMP Plug in a crimped

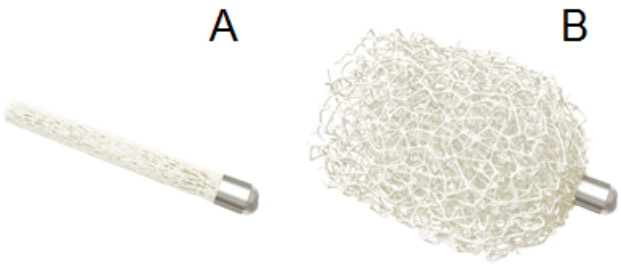


Figure 7.1: The IMPEDE-FX Embolization Plug in its crimped (a) and expanded (b) state

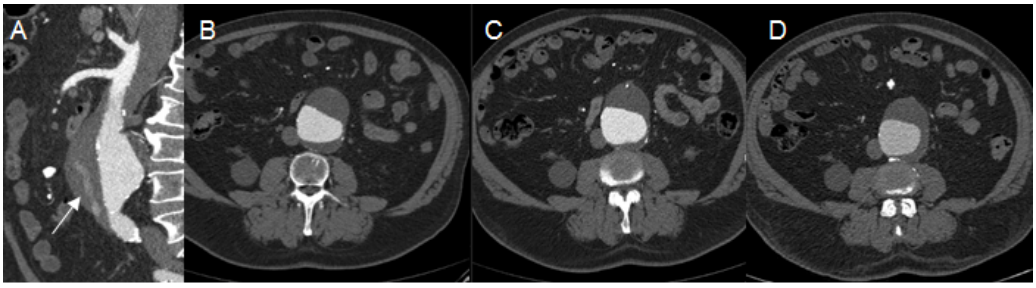


Figure 7.2: CT angiography of the post-dissection infrarenal aneurysm in the sagittal plane showing the filling of the false lumen (a), transversal slide of a CT angiography of the post-dissection infrarenal aneurysm before treatment (b), one month postoperatively (c) and 8 months postoperatively (d).

state, and self-expand by exposure to an aqueous environment and body temperature (fig. 7.1a and 7.1b). The largest IMPEDE-FX Embolization Plug has an expanded diameter of 12 mm. SMP has been proposed as a suitable biomaterial for endovascular embolization applications due to their capability of shape recovery [98] and the interconnected, large surface area porosity [99]. This interconnected porosity of the SMP serves as a scaffold for blood flow, thrombus formation, and tissue healing by supporting rapid formation of small interconnected clots throughout its structure. SMP is considered to provide a stable occlusion by promoting fast conversion to organized thrombus, followed by collagen deposition without chronic active inflammation. The SMP Plug offers a high embolic material volume and inherent 100% packing density. To avoid recanalization, the packing density is an important factor [100]. The IMPEDE-FX embolization plug received CE Mark approval in 2018 in Europe. In this report, we describe a case of an infrarenal post-dissection aneurysm that was successfully embolized using multiple IMPEDE-FX embolization plugs.

7.3 Case report

In 2010, a 69-year-old patient was treated with a mitral valve replacement, complicated by a Crawford type A dissection. In January 2019 he presented with an enlarging infrarenal post-dissection aneurysm with a maximum diameter of 81 mm. In 2010 this diameter was 52 mm. Contrast enhanced computed tomography (CTA) scanning showed a post-dissection infrarenal aneurysm without remaining proximal filling through the entry tear. The original dissection originated from the aortic arch to the level of the abdominal aorta and extended to the right CIA. Diameters at the thoracic level were below the threshold for intervention. The celiac trunk, superior mesenteric artery and left renal artery derived from the true lumen, while the left renal artery was occluded. There was filling of the false lumen of the aneurysm through the inferior mesenteric artery (IMA) and the right external iliac artery (fig. 7.2a). The maximum diameter of the aneurysm was 81 mm, with a true lumen diameter of 56 mm (fig. 7.2b).

After ample consideration and informed consent the patient was scheduled for embolization of the IMA and filling of the false lumen, using IMPEDE-FX embolization plug. Patient was operated under general anesthesia and antibiotic prophylaxis. After placement of a 5-F sheath a blowback angiography was performed (fig. 7.3a). Subsequently, the false lumen was cannulated. Angiography showed the false lumen and the IMA (fig. 7.3b). Subsequently a 5-F sheath was advanced into the IMA, and it was embolized using a 5x80 mm Interlock-18 microcoil (Boston Scientific, Marlborough, MA, USA) to prevent distal migration of the IMPEDE-FX Plug, just before its first bifurcation, and proximal of Riolan to guarantee collateral flow. Then an IMPEDE-FX-12 Embolization Plug was placed in the orifice of the IMA followed by 6 other IMPEDE-FX-12 Embolization Plugs, that filled the entire false lumen (fig. 7.3d).

Afterwards, the true lumen was cannulated and, in order to secure the entry in the iliac artery, a 11mmx39 balloon-expandable covered stent (GORE® VIABAHN® VBX Balloon Expandable Endoprosthesis, W.L.Gore and associates, Flagstaff, AZ, USA) was placed in external iliac artery. Completion angiography showed a fully excluded false lumen with patent flow through the true lumen. The most distal plug, however, seemed to have moved distally, indicating that the re-entry was not fully covered. Therefore, the balloon-expandable covered stent was extended distally to the level of the deep inferior epigastric artery with a good result. The access site was closed using an Angio-Seal (Terumo Interventional Systems, Somerset, NJ, USA) vascular closure device. The post-procedural course was uneventful and patient was discharged at the 1st postoperative day.
At one month postoperatively a CTA showed a fully thrombosed false lumen. The diameter of the aneurysm decreased from 81 mm to 79 mm (fig. 7.2c). At 8 months another CTA was performed confirming the full thrombosis of the false lumen. The total diameter of the false lumen remained stable (fig. 7.2d). Furthermore, remodeling of the true lumen was evident. The shape of the true lumen had changed as the thrombus load at the left lateral side was increased

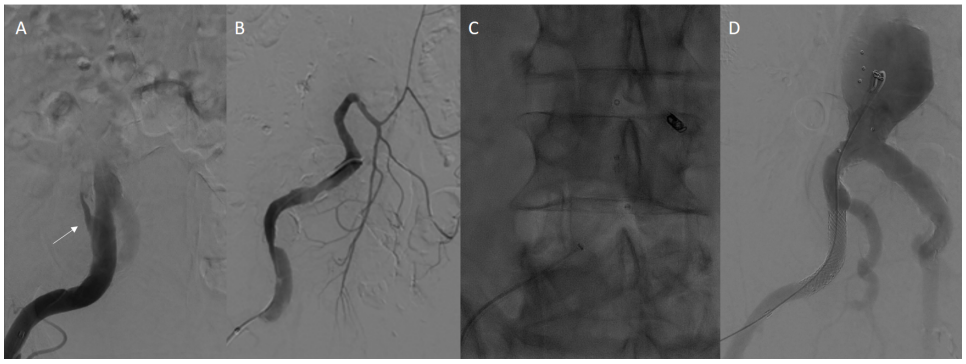


Figure 7.3: Procedural angiography showing the filling right iliac artery through the iliac entry tear (a), the filling of the false lumen and the IMA (b), the position of the SMP plugs (c), and completion angiography showing filling of the true lumen with complete obliteration of the false lumen (d).

(fig. 7.2). The maximum diameters of the true lumen decreased from 56 mm, to 56 mm and 52 mm preoperatively at 1 month and 8 months, respectively. The volume of the true lumen showed a similar decrease, with a preoperative volume of 102.4 cm^3, and a volume of 109.2 cm^3 and 87.5 cm^3 at 1 and 8 months, respectively.

7.4 Discussion

This case demonstrates the successful use of IMPEDE-FX embolization Plugs to obliterate a false lumen of an infrarenal post-dissection aneurysm. This method could provide an alternative technique for endovascular embolization of larger false lumen in patients with an aortic dissection, also in the thoracic area. The ease of use, the option to use multiple plugs, controlled release and the off-the-shelve availability could facilitate treatment of a wide range of patients. The device holds promise for patients with a not-thrombosed false lumen as well as patients with a type II endoleak after EVAR requiring treatment. In addition, the polymer plugs cause only minimal radiographic artifacts and has a favorable property of rapid clot maturation.

When using conventional coils a recanalization rate up to 20% has been described [101].The difference between conventional coils and the IMPEDE-FX embolization Plug is that conventional coils only induces thrombosis of fresh thrombus, while the latter provides a scaffold for tissue ingrowth. The plugs thus aim to minimize time to thrombus maturation by promoting initial clotting of blood within the scaffold, which will be replaced by connective tissue over time and this reduces the risk of recanalization. The efficacy of the device was previously verified in-vitro by blood flow studies that established affinity for thrombus formation and blood penetration throughout the foam and by an ultrasound phantom that showed flow stagnation and diversion of flow to collateral pathways [102]. In a porcine model, Rodriquez et al. [98, 103] showed significant connective tissue infiltration throughout the implant of SMP foams, which caused complete and stable occlusion of treated intracranial aneurysms. The material is also shown to be less inflammatory compared to traditionally used suture materials, and it encouraged collagen formation, neovascularization, lack of fibrin and a complete endothelial layer across the ostium of the aneurysm after 90 days of implantation [98]. Another advantage of the IMPEDE-FX embolization Plugs is that the undergo a slow degradation. In an experimental setting most of the material is degraded at 180 days after implantation[104].

In the current patient we have decided to leave the true lumen untreated, as both the diameter and volume decreased postoperatively. The maximum diameter at 8 months postoperatively was 52 mm and therefore is not eligible for surgical treatment. Endovascular treatment was considered to be challenging due to an extreme elliptical shape of the infrarenal neck. Besides diameter, the shape of the true lumen also changed and the volume decreased by about 15%, after a minor increase at the early postoperative CT scan, which may be explained by the decrease of pressure in the false lumen.

In conclusion, this case suggest that IMPEDE-FX embolization plugs can be used successfully to embolize a false lumen of a post-dissection aneurysm, also with a larger diameter. Confirmatory prospective trials on patients with non-thrombosed false lumina are indicated.

Bibliography

[1] R. Greenhalgh, J. Powell, L. Brown, and S. Thompson, "Endovascular repair of abdominal aortic aneurysm – Authors' reply," *N Engl J Med*, vol. 358, no. 5, pp. 494–501, 2008.

[2] U. K. Sampson, P. E. Norman, F. G. R. Fowkes, V. Aboyans, Y. Song, F. E. Harrell, M. H. Forouzanfar, M. Naghavi, J. O. Denenberg, M. M. McDermott, M. H. Criqui, G. A. Mensah, M. Ezzati, and C. Murray, "Estimation of global and regional incidence and prevalence of abdominal aortic aneurysms 1990 to 2010," *Global Heart*, vol. 9, no. 1, pp. 159–170, 2014. [Online]. Available: http://dx.doi.org/10.1016/j.gheart.2013.12.009

[3] D. Sidloff, P. Stather, N. Dattani, M. Bown, J. Thompson, R. Sayers, and E. Choke, "Aneurysm global epidemiology study public health measures can further reduce abdominal aortic aneurysm mortality," *Circulation*, vol. 129, no. 7, pp. 747–753, 2014.

[4] S. Svensjö, M. Björck, M. Gürtelschmid, K. Djavani Gidlund, A. Hellberg, and A. Wanhainen, "Low prevalence of abdominal aortic aneurysm among 65-year-old swedish men indicates a change in the epidemiology of the disease," *Circulation*, vol. 124, no. 10, pp. 1118–1123, 2011.

[5] F. A. Lederle, S. E. Wilson, G. R. Johnson, D. B. Reinke, F. N. Littooy, C. W. Acher, D. J. Ballard, L. M. Messina, I. L. Gordon, E. P. Chute, W. C. Krupski, and D. Bandyk, "Immediate repair compared with surveillance of small abdominal aortic aneurysms," *The New England Journal of Medicine*, vol. 346, no. 19, pp. 1437–1444, 2002.

[6] J. J. Reimerink, M. J. Van Der Laan, M. J. Koelemay, R. Balm, and D. A. Legemate, "Systematic review and meta-analysis of population-based mortality from ruptured abdominal aortic aneurysm," *British Journal of Surgery*, vol. 100, no. 11, pp. 1405–1413, 2013.

[7] M. J. Bown, A. J. Sutton, P. R. Bell, and R. D. Sayers, "A meta-analysis of 50 years of ruptured abdominal aortic aneurysm repair," *British Journal of Surgery*, vol. 89, no. 6, pp. 714–730, 2002.

[8] A. B. Newman, A. M. Arnold, G. L. Burke, D. H. O'Leary, and T. A. Manolio, "Cardiovascular disease and mortality in older adults with small abdominal aortic aneurysms detected by ultrasonography: The cardiovascular health study," *Annals of Internal Medicine*, vol. 134, no. 3, 2001.

[9] M. F. Bath, V. J. Gokani, D. A. Sidloff, L. R. Jones, E. Choke, R. D. Sayers, and M. J. Bown, "Systematic review of cardiovascular disease and cardiovascular death in patients with a small abdominal aortic aneurysm," *British Journal of Surgery*, vol. 102, no. 8, pp. 866–872, 2015.

[10] I. O. Haefliger, J. Flammer, J. L. Bény, and T. F. Lüscher, "Endothelium-dependent vasoactive modulation in the ophthalmic circulation," *Progress in Retinal and Eye Research*, vol. 20, no. 2, pp. 209–225, 2001.

[11] G. Brevetti, A. Silvestro, V. Schiano, and M. Chiariello, "Endothelial Dysfunction and Cardiovascular Risk Prediction in Peripheral Arterial Disease: Additive Value of Flow-Mediated Dilation to Ankle-Brachial Pressure Index," *Circulation*, vol. 108, no. 17, pp. 2093–2098, 2003.

[12] N. Gokce, J. F. Keaney, L. M. Hunter, M. T. Watkins, Z. S. Nedeljkovic, J. O. Menzoian, and J. A. Vita, "Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease," *Journal of the American College of Cardiology*, vol. 41, no. 10, pp. 1769–1775, 2003.

[13] A. C. Van Mil, Y. Hartman, F. Van Oorschot, A. Heemels, N. Bax, E. A. Dawson, N. Hopkins, M. T. Hopman, D. J. Green, D. L. Oxborough, and D. H. Thijssen, "Correlation of carotid artery reactivity with cardiovascular risk factors and coronary artery vasodilator responses in asymptomatic, healthy volunteers," *Journal of Hypertension*, vol. 35, no. 5, pp. 1026–1034, 2017.

[14] A. C. van Mil, S. Pouwels, J. Wilbrink, M. C. Warlé, and D. H. Thijssen, "Carotid Artery Reactivity Predicts Events in Peripheral Arterial Disease Patients," *Annals of surgery*, vol. 269, no. 4, pp. 767–773, 2017.

[15] P. A. N. Chandraratna, K. Wijegunaratne, K. F. Farag, A. R. Nimalasuriya, and S. J. Mathews, "Changes in abdominal aortic diameter in response to the cold pressor test and nitroglycerin: A new noninvasive model for the assessment of endothelial-dependent and endothelial-independent vascular relaxation," *Echocardiography*, vol. 26, no. 10, pp. 1211–1216, 2009.

[16] Z.-H. Sun, "Abdominal aortic aneurysm: Treatment options, image visualizations and follow-up procedures," *Journal of Geriatric Cardiology*, vol. 9, no. 1, pp. 49–60, 2012.

[17] A. Wanhainen, F. Verzini, I. Van Herzeele, E. Allaire, M. Bown, T. Cohnert, F. Dick, J. van Herwaarden, C. Karkos, M. Koelemay, T. Kölbel, I. Loftus, K. Mani, G. Melissano, J. Powell, Z. Szeberin, ESVS Guidelines Committee, G. J. de Borst, N. Chakfe, S. Debus, R. Hinchliffe, S. Kakkos, I. Koncar, P. Kolh, J. S. Lindholt, M. de Vega, F. Vermassen, Document reviewers, M. Björck, S. Cheng, R. Dalman, L. Davidovic, K. Donas, J. Earnshaw, H. H. Eckstein, J. Golledge, S. Haulon, T. Mastracci, R. Naylor, J. B. Ricco, and H. Verhagen, "European Society for Vascular Surgery (ESVS) 2019 Clinical Practice Guidelines on the Management of Abdominal Aorto-iliac Artery Aneurysms," *European Journal of Vascular and Endovascular Surgery*, vol. 57, no. 1, pp. 1–97, 2018. [Online]. Available: http://dx.doi.org/10.1016/j.ejvs.2018.09.020

[18] D. Carino, T. P. Sarac, B. A. Ziganshin, and J. A. Elefteriades, "Abdominal Aortic Aneurysm: Evolving Controversies and Uncertainties," *International Journal of Angiology*, vol. 27, no. 2, pp. 58–80, 2018.

[19] A. Wanhainen, D. Bergqvist, K. Boman, T. K. Nilsson, J. Rutegård, and M. Björck, "Risk factors associated with abdominal aortic aneurysm: A population-based study with historical and current data," *Journal of Vascular Surgery*, vol. 41, no. 3, pp. 390–396, 2005.

[20] E. Jahangir, L. Lipworth, T. L. Edwards, E. K. Kabagambe, M. T. Mumma, G. A. Mensah, S. Fazio, W. J. Blot, and U. K. Sampson, "Smoking, sex, risk factors and abdominal aortic aneurysms: A prospective study of 18 782 persons aged above 65 years in the southern community cohort study," *Journal of Epidemiology and Community Health*, vol. 69, no. 5, pp. 481–488, 2015.

[21] M. Sprynger, M. Willems, H. Van Damme, B. Drieghe, J. C. Wautrecht, and M. Moonen, "Screening Program of Abdominal Aortic Aneurysm," *Angiology*, 2019.

[22] C. M. Wahlgren, E. Larsson, P. K. Magnusson, R. Hultgren, and J. Swedenborg, "Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population," *Journal of Vascular Surgery*, vol. 51, no. 1, pp. 3–7, 2010. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2009.08.036

[23] J. M. Ruddy, J. A. Jones, F. G. Spinale, and J. S. Ikonomidis, "Regional heterogeneity within the aorta: Relevance to aneurysm disease," *Journal of Thoracic and Cardiovascular Surgery*, vol. 136, no. 5, pp. 1123–1130, 2008. [Online]. Available: http://dx.doi.org/10.1016/j.jtcvs.2008.06.027

[24] G. Ailawadi, J. L. Eliason, and G. R. Upchurch Jr, "Current Concepts for the Pathogenesis of Abdominal Aortic Aneurysms," *Journal of Vascular Surgery*, vol. 38, no. 3, pp. 584–8, 2003.

[25] A. Busch, E. Hartmann, C. Grimm, S. Ergün, R. Kickuth, C. Otto, R. Kellersmann, and U. Lorenz, "Heterogeneous histomorphology, yet homogeneous vascular smooth muscle cell dedifferentiation, characterize human aneurysm disease," *Journal of Vascular Surgery*, vol. 66, no. 5, pp. 1553–1564, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2016.07.129

[26] M. Cecelja and P. Chowienczyk, "Role of arterial stiffness in cardiovascular disease," *JRSM Cardiovascular Disease*, vol. 1, no. 4, pp. 1–10, 2013.

[27] F. Parkinson, S. Ferguson, P. Lewis, I. M. Williams, and C. P. Twine, "Rupture rates of untreated large abdominal aortic aneurysms in patients unfit for elective repair," *Journal of Vascular Surgery*, vol. 61, no. 6, pp. 1606–1612, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2014.10.023

[28] J. D. Blankensteijn, "De behandeling van het aneurysma van de aorta abdominalis | Nederlands Tijdschrift voor Geneeskunde," vol. 147, no. 14, pp. 1–6, 2003. [Online]. Available: https://www.ntvg.nl/artikelen/de-behandeling-van-het-aneurysma-van-de-aorta-abdominalis/volledig

[29] A. Tzani, I. P. Doulamis, I. Katsaros, E. Martinou, D. Schizas, and K. P. Economopoulos, "Mortality after endovascular treatment of infrarenal abdominal aortic aneurysms – the newer the better?" *Vasa*, vol. 47, pp. 187–196, 2018.

[30] S. S. Bahia, P. J. Holt, D. Jackson, B. O. Patterson, R. J. Hinchliffe, M. M. Thompson, and A. Karthikesalingam, "Systematic Review and Meta-analysis of Long-term survival after Elective Infrarenal Abdominal Aortic Aneurysm Repair 1969-2011: 5 Year Survival Remains Poor Despite Advances in Medical Care and Treatment Strategies," *European Journal of Vascular and Endovascular Surgery*, vol. 50, no. 3, pp. 320–330, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.ejvs.2015.05.004

[31] F. B. Gonçalves, K. H. Ultee, S. E. Hoeks, R. J. Stolker, and H. J. Verhagen, "Life expectancy and causes of death after repair of intact and ruptured abdominal aortic aneurysms Presented in the Plenary Rapid Pace Session at the 2015 Vascular Annual Meeting of the Society for Vascular Surgery, Chicago, Ill, June 17-20, 2015." *Journal of Vascular Surgery*, vol. 63, no. 3, pp. 610–616, 2016. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2015.09.030

[32] F. A. Lederle, J. A. Freischlag, T. C. Kyriakides, J. S. Matsumura, F. T. Padberg, T. R. Kohler, P. Kougias, J. M. Jean-Claude, D. F. Cikrit, and K. M. Swanson, "Long-term comparison of endovascular and open repair of abdominal aortic aneurysm," *New England Journal of Medicine*, vol. 367, no. 21, pp. 1988–1997, 2012.

[33] J. P. Becquemin, J. C. Pillet, F. Lescalie, M. Sapoval, Y. Goueffic, P. Lermusiaux, E. Steinmetz, and J. Marzelle, "A randomized controlled trial of endovascular aneurysm repair versus open surgery for abdominal aortic aneurysms in low- to moderate-risk patients," *Journal of Vascular Surgery*, vol. 53, no. 5, pp. 1167–1173, 2011. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2010.10.124

[34] P. P. Goodney, D. Tavris, F. L. Lucas, T. Gross, E. S. Fisher, and S. R. Finlayson, "Causes of late mortality after endovascular and open surgical repair of infrarenal abdominal aortic aneurysms," *Journal of Vascular Surgery*, vol. 51, no. 6, pp. 1340–1347, 2010. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2010.01.054

[35] L. C. Brown, S. G. Thompson, R. M. Greenhalgh, and J. T. Powell, "Incidence of cardiovascular events and death after open or endovascular repair of abdominal aortic aneurysm in the randomized EVAR trial 1," *British Journal of Surgery*, vol. 98, no. 7, pp. 935–942, 2011.

[36] J. A. N. Dorresteijn, F. L. J. Visseren, A. M. J. Wassink, M. J. A. Gondrie, E. W. Steyerberg, P. M. Ridker, N. R. Cook, and Y. van der Graaf, "Development and validation of a prediction rule for recurrent vascular events based on a cohort study of patients with arterial disease: the SMART risk score," *Heart*, vol. 99, no. 12, pp. 866–872, 2013.

[37] S. S. Bahia, A. Vidal-Diez, S. R. Seshasai, I. Shpitser, J. R. Brownrigg, B. O. Patterson, K. K. Ray, P. J. Holt, M. M. Thompson, and A. Karthikesalingam, "Cardiovascular risk prevention and all-cause mortality in primary care patients with an abdominal aortic aneurysm," *British Journal of Surgery*, vol. 103, no. 12, pp. 1626–1633, 2016.

[38] W. Karrowni, S. Dughman, G. P. Hajj, and F. J. Miller, "Statin therapy reduces growth of abdominal aortic aneurysms," *Journal of Investigative Medicine*, vol. 59, no. 8, pp. 1239–1243, 2011.

[39] Y. M. Ulrich-Lai and J. P. Herman, "Neural regulation of endocrine and autonomic stress responses," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 397–409, 2009.

[40] A. W. Tank and D. L. Wong, "Peripheral and central effects of circulating catecholamines," *Comprehensive Physiology*, vol. 5, no. 1, pp. 1–15, 2015.

[41] N. Schneiderman, G. Ironson, and S. D. Siegel, "Stress and Health: Psychological, Behavioral, and Biological Determinants," *Annual Review of Clinical Psychology*, vol. 1, no. 1, pp. 607–628, 2005.

[42] R. Duncko, L. Johnson, K. Merikangas, and C. Grillon, "Working memory performance after acute exposure to the cold pressor stress in healthy volunteers," *Neurobiology of Learning and Memory*, vol. 91, no. 4, pp. 377–381, 2009. [Online]. Available: http://dx.doi.org/10.1016/j.nlm.2009.01.006

[43] A. M. Rutchick and M. L. Slepian, "Handling Ibuprofen Increases Pain Tolerance and Decreases Perceived Pain Intensity in a Cold Pressor Test," *PLoS ONE*, vol. 8, no. 3, pp. 1–5, 2013.

[44] C. L. Von Baeyer, T. Piira, C. T. Chambers, M. Trapanotto, and L. K. Zeltzer, "Guidelines for the cold pressor task as an experimental pain stimulus for use with children," *Journal of Pain*, vol. 6, no. 4, pp. 218–227, 2005.

[45] K. D. Monahan, R. P. Feehan, L. I. Sinoway, and Z. Gao, "Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: Effect of ageing," *Journal of Physiology*, vol. 591, no. 11, pp. 2937–2947, 2013.

[46] R. J. Northcote and M. B. Cooke, "How useful are the cold pressor test and sustained isometric handgrip exercise with radionuclide ventriculography in the evaluation of patients with coronary artery disease?" *Heart*, vol. 57, no. 4, pp. 319–328, 1987.

[47] J. L. Wirch, L. A. Wolfe, T. L. Weissgerber, and G. A. Davies, "Cold presser test protocol to evaluate cardiac autonomic function," *Applied Physiology, Nutrition and Metabolism*, vol. 31, no. 3, pp. 235–243, 2006.

[48] D. U. Silverthorn and J. Michael, "Cold stress and the cold pressor test," *American Journal of Physiology - Advances in Physiology Education*, vol. 37, no. 1, pp. 93–96, 2013.

[49] E. G. Nabel, P. Ganz, J. B. Gordon, R. W. Alexander, D. Ph, and A. P. Selwyn, "Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold test," *Circulation*, vol. 77, no. 1, pp. 43–52, 1988.

[50] A. J. Miller, J. Cui, J. C. Luck, L. I. Sinoway, and M. D. Muller, "Age and sex differences in sympathetic and hemodynamic responses to hypoxia and cold pressor test," *Physiological Reports*, vol. 7, no. 2, pp. 1–8, 2019.

[51] L. Mourot, M. Bouhaddi, and J. Regnard, "Effects of the cold pressor test on cardiac autonomic control in normal subjects," *Physiological Research*, vol. 58, no. 1, pp. 83–91, 2009.

[52] A. M. Zeiher, H. Drexler, H. Wollschlaeger, B. Saurbier, and H. Just, "Coronary vasomotion in response to sympathetic stimulation in humans: Importance of the functional integrity of the endothelium," *Journal of the American College of Cardiology*, vol. 14, no. 5, pp. 1181–1190, 1989.

[53] C. Seiler, O. M. Hess, M. Buechi, T. M. Suter, and H. P. Krayenbuehl, "Influence of serum cholesterol and other coronary risk factors on vasomotion of angiographically normal coronary arteries," *Circulation*, vol. 88, no. 5, pp. 2139–2148, 1993.

[54] V. Schächinger, M. B. Britten, and A. M. Zeiher, "Prognostic impact of coronary vasodilator dysfunction on adverse long- term outcome of coronary heart disease," *Circulation*, vol. 101, no. 16, pp. 1899–1906, 2000.

[55] A. Nitenberg, D. Chemla, and I. Antony, "Epicardial coronary artery constriction to cold pressor test is predictive of cardiovascular events in hypertensive patients with angiographically normal coronary arteries and without other major coronary risk factor," *Atherosclerosis*, vol. 173, no. 1, pp. 115–123, 2004.

[56] M. Rubenfire, S. Rajagopalan, and L. Mosca, "Carotid artery vasoreactivity in response to sympathetic stress correlates with coronary disease risk and is independent of wall thickness," *Journal of the American College of Cardiology*, vol. 36, no. 7, pp. 2192–2197, 2000. [Online]. Available: http://dx.doi.org/10.1016/S0735-1097(00)01021-4

[57] Y. Sugiyama, T. Matsukawa, A. S. Shamsuzzaman, H. Okada, T. Watanabe, and T. Mano, "Delayed and diminished presser response to muscle sympathetic nerve activity in the elderly," *Journal of Applied Physiology*, vol. 80, no. 3, pp. 869–875, 1996.

[58] A. V. Ng, R. Callister, D. G. Johnson, and D. R. Seals, "Age and Gender Influence Muscle Sympathetic Nerve Activity at Rest in Healthy Humans," *Hypertension*, vol. 21, pp. 498–503, 1993.

[59] B. Gribbin, T. G. Pickering, P. Sleight, and R. Peto, "Effect of age and high blood pressure on baroreflex sensitivity in man." *Circulation research*, vol. 29, no. 4, pp. 424–431, 1971.

[60] T. G. Pickering, B. Gribbin, and P. Sleight, "Comparison of the reflex heart rate response to rising and falling arterial pressure in man," *Cardiovascular Research*, vol. 6, no. 3, pp. 277–283, 1972.

[61] J. H. Fleisch, "Age-related changes in the sensitivity of blood vessels to drugs," *Pharmac. Ther.*, vol. 8, pp. 477–487, 1980.

[62] R. S. Tuttle, "Age-related changes in the sensitivity of rat aortic strips to norepinephrine and associated chemical and structural alterations." *Journal of gerontology*, vol. 21, no. 4, pp. 510–516, 1966.

[63] F. Blandini, E. Martignoni, G. V. Melzi D'Eril, L. Biasio, G. Sances, C. Lucarelli, V. Rizzo, A. Costa, and G. Nappi, "Free plasma catecholamine levels in healthy subjects: A basal and dynamic study. The influence of age," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 52, no. 1, pp. 9–17, 1992.

[64] Z. Gao, T. E. Wilson, R. C. Drew, J. Ettinger, and K. D. Monahan, "Altered coronary vascular control during cold stress in healthy older adults," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 302, no. 1, pp. 312–318, 2012.

[65] D. C. Barratt, B. B. Ariff, K. N. Humphries, S. A. McG Thom, and A. D. Hughes, "Reconstruction and quantification of the carotid artery bifurcation from 3-D ultrasound images," *IEEE Transactions on Medical Imaging*, vol. 23, no. 5, pp. 567–583, 2004.

[66] D. F. Leotta, M. Paun, K. W. Beach, T. R. Kohler, R. E. Zierler, and D. E. Strandness, "Measurement of abdominal aortic aneurysms with three-dimensional ultrasound imaging: Preliminary report," *Journal of Vascular Surgery*, vol. 33, no. 4, pp. 700–707, 2001.

[67] A. AbuRahma and D. Bandyk, Eds., *Noninvasive Vascular Diagnosis: A Practical Guide to Therapy*, 3rd ed. London: Springer-Verlag, 2013.

[68] C. Leys, C. Ley, O. Klein, P. Bernard, and L. Licata, "Journal of Experimental Social Psychology Detecting outliers : Do not use standard deviation around the mean, use absolute deviation around the median," *Experimental Social Psychology*, pp. 4–6, 2013.

[69] H. Abdul-Hussien, R. Hanemaaijer, R. Kleemann, B. F. Verhaaren, J. H. van Bockel, and J. H. Lindeman, "The pathophysiology of abdominal aortic aneurysm growth: Corresponding and discordant inflammatory and proteolytic processes in abdominal aortic and popliteal artery aneurysms," *Journal of Vascular Surgery*, vol. 51, no. 6, pp. 1479–1487, 2010. [Online]. Available: http://dx.doi.org/10.1016/j.jvs.2010.01.057

[70] D. H. Thijssen, M. A. Black, K. E. Pyke, J. Padilla, G. Atkinson, R. A. Harris, B. Parker, M. E. Widlansky, M. E. Tschakovsky, and D. J. Green, "Assessment of flow-mediated dilation in humans: A methodological and physiological guideline," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 300, no. 1, pp. 2–12, 2011.

[71] A. Mahmud and J. Feely, "Divergent effect of acute and chronic alcohol on arterial stiffness," *American Journal of Hypertension*, vol. 15, no. 3, pp. 240–243, 2002.

[72] L. M. Title, P. M. Cummings, K. Giddens, and B. A. Nassar, "Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: An effect prevented by vitamins C and E," *Journal of the American College of Cardiology*, vol. 36, no. 7, pp. 2185–2191, 2000. [Online]. Available: http://dx.doi.org/10.1016/S0735-1097(00)00980-3

[73] E. A. Dawson, G. P. Whyte, M. A. Black, H. Jones, N. Hopkins, D. Oxborough, D. Gaze, R. E. Shave, M. Wilson, K. P. George, and D. J. Green, "Changes in vascular and cardiac function after prolonged strenuous exercise in humans," *Journal of Applied Physiology*, vol. 105, no. 5, pp. 1562–1568, 2008.

[74] A. Mahmud and J. Feely, "Effect of smoking on arterial stiffness and pulse pressure amplification," *Hypertension*, vol. 41, no. 1, pp. 183–187, 2003.

[75] R. A. Vogel, M. C. Corretti, and G. D. Plotnick, "Effect of a single high-fat meal on endothelial function in healthy subjects," *American Journal of Cardiology*, vol. 79, no. 3, pp. 350–354, 1997.

[76] T. Neunteufl, U. Priglinger, S. Heher, M. Zehetgruber, G. Söregi, S. Lehr, K. Huber, G. Maurer, F. Weidinger, and K. Kostner, "Effects of vitamin E on chronic and acute endothelial dysfunction in smokers," *Journal of the American College of Cardiology*, vol. 35, no. 2, pp. 277–283, 2000.

[77] H. Westling, L. Jansson, B. Jonson, and R. Nilsén, "Vasoactive drugs and elastic properties of human arteries in vivo, with special reference to the action of nitroglycerine," *European Heart Journal*, vol. 5, no. 8, pp. 609–616, 1984.

[78] P. B. Dobrin and A. A. Rovick, "Influence of vascular smooth muscle on contractile mechanics and elasticity of arteries." *The American journal of physiology*, vol. 217, no. 6, pp. 1644–1651, 1969.

[79] M. Gerová and J. Gero, "Tension of the Lower Abdominal Aorta as Influenced by Sympathetic Stimulation," *Experientia*, vol. 26, no. 12, pp. 1318–9, 1970.

[80] B. Sonesson, E. Vernersson, F. Hansen, and T. Länne, "Influence of sympathetic stimulation on the mechanical properties of the aorta in humans," *Acta Physiologica Scandinavica*, vol. 159, no. 2, pp. 139–145, 1997.

[81] S. Makita, A. Ohira, R. Tachieda, S. Itoh, Y. Moriai, H. Niinuma, M. Nakamura, and K. Hiramori, "Dilation and reduced distensibility of carotid artery in patients with abdominal aortic aneurysms," *American Heart Journal*, vol. 140, no. 2, pp. 297–302, 2000.

[82] L. P. Harter, B. H. Gross, P. W. Callen, and R. A. Barth, "Ultrasonic evaluation of abdominal aortic thrombus," *Journal of Ultrasound in Medicine*, vol. 1, no. 8, pp. 315–318, 1982.

[83] P. Stoica and R. Moses, *Spectral Analysis of Signals*, T. Robbins, Ed. Upper Saddle River: Prentice Hall, 2005.

[84] V. E. Noble, B. Nelson, and A. N. Sutingco, *Manual of emergency and critical care ultrasound*, 2007.

[85] S. Dort, "Vector flow mapping using plane-wave ultrasound imaging," pp. 6–49, 2013. [Online]. Available: http://www.biomecardio.com/files/thesis$_M Sc_S D13.pdf$

[86] K. A. Carmody, C. L. Moore, and D. Feller-Kopman, "Probe Selection, Machine Controls, and Equipment," in *Handbook of Critical Care & Emergency Ultrasound*, 1st ed. McGraw-Hill Education / Medical, 2011, ch. 4, Probe S.

[87] A. Scorza, S. Conforto, C. D'Anna, and S. Sciuto, "A Comparative Study on the Influence of Probe Placement on Quality Assurance Measurements in B-mode Ultrasound by Means of Ultrasound Phantoms," *The Open Biomedical Engineering Journal*, vol. 9, no. 1, pp. 164–178, 2015.

[88] S. Li, W. N. McDicken, and P. R. Hoskins, "Blood vessel diameter measurement by ultrasound," *Physiological Measurement*, vol. 14, no. 3, pp. 291–297, 1993.

[89] T. Thomaes, M. Thomis, S. Onkelinx, W. Coudyzer, V. Cornelissen, and L. Vanhees, "Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients," *BMC Medical Imaging*, vol. 12, no. 1, p. 7, 2012. [Online]. Available: http://www.biomedcentral.com/1471-2342/12/7

[90] W. Nijholt, H. Jager-Wittenaar, I. S. Raj, C. P. van der Schans, and H. Hobbelen, "Reliability and validity of ultrasound to estimate muscles: A comparison between different transducers and parameters," *Clinical Nutrition ESPEN*, vol. 35, pp. 146–152, 2020.

[91] Q. M. Ghulam, K. K. Bredahl, L. Lönn, L. Rouet, H. H. Sillesen, and J. P. Eiberg, "Follow-up on Small Abdominal Aortic Aneurysms Using Three Dimensional Ultrasound: Volume Versus Diameter," *European Journal of Vascular and Endovascular Surgery*, vol. 54, no. 4, pp. 439–445, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.ejvs.2017.06.016

[92] F. Rohlffs, K. Spanos, N. Tsilimparis, E. S. Debus, and T. Kölbel, "Techniques and outcomes of false lumen embolization in chronic type B aortic dissection," *Journal of Cardiovascular Surgery*, vol. 59, no. 6, pp. 784–788, 2018.

[93] J. M. Song, S. D. Kim, J. H. Kim, M. J. Kim, D. H. Kang, J. B. Seo, T. H. Lim, J. W. Lee, M. G. Song, and J. K. Song, "Long-Term Predictors of Descending Aorta Aneurysmal Change in Patients With Aortic Dissection," *Journal of the American College of Cardiology*, vol. 50, no. 8, pp. 799–804, 2007.

[94] S. C. Hofferberth, I. K. Nixon, and P. J. Mossop, "Aortic false lumen thrombosis induction by embolotherapy (AFTER) following endovascular repair of aortic dissection," *Journal of Endovascular Therapy*, vol. 19, no. 4, pp. 538–545, 2012.

[95] J. Idrees, E. E. Roselli, S. Shafii, J. Reside, and B. W. Lytle, "Outcomes after false lumen embolization with covered stent devices in chronic dissection," *Journal of Vascular Surgery*, vol. 60, no. 6, pp. 1507–1513, 2014.

[96] T. Kölbel, C. Lohrenz, A. Kieback, H. Diener, E. S. Debus, and A. Larena-Avellaneda, "Distal false lumen occlusion in aortic dissection with a homemade extra-large vascular plug: The candy-plug technique," *Journal of Endovascular Therapy*, vol. 20, no. 4, pp. 484–489, 2013.

[97] "IMPEDE - FX Embolization Plug Instructions for Use, Shape memory medical," 2019.

[98] J. N. Rodriguez, F. J. Clubb, T. S. Wilson, M. W. Miller, T. W. Fossum, J. Hartman, E. Tuzun, P. Singhal, and D. J. Maitland, "In vivo response to an implanted shape memory polyurethane foam in a porcine aneurysm model," *Journal of Biomedical Materials Research - Part A*, vol. 102, no. 5, pp. 1231–1242, 2014.

[99] P. Singhal, J. N. Rodriguez, W. Small, S. Eagleston, J. Van De Water, D. J. Maitland, and T. S. Wilson, "Ultra low density and highly crosslinked biocompatible shape memory polyurethane foams," *Journal of Polymer Science, Part B: Polymer Physics*, vol. 50, no. 10, pp. 724–737, 2012.

[100] T. Yasumoto, K. Osuga, H. Yamamoto, Y. Ono, M. Masada, K. Mikami, D. Kanamori, M. Nakamura, K. Tanaka, T. Nakazawa, H. Higashihara, N. Maeda, and N. Tomiyama, "Long-term outcomes of coil packing for visceral aneurysms: Correlation between packing density and incidence of coil compaction or recanalization," *Journal of Vascular and Interventional Radiology*, vol. 24, no. 12, pp. 1798–1807, 2013. [Online]. Available: http://dx.doi.org/10.1016/j.jvir.2013.04.030

[101] J. Enriquez, S. Javadi, R. Murthy, J. Ensor, A. Mahvash, M. E. Abdelsalam, D. C. Madoff, M. J. Wallace, and R. Avritscher, "Gastroduodenal artery recanalization after transcatheter fibered coil embolization for prevention of hepaticoenteric flow: Incidence and predisposing technical factors in 142 patients," *Acta Radiologica*, vol. 54, no. 7, pp. 790–794, 2013.

[102] T. L. Landsman, R. L. Bush, A. Glowczwski, J. Horn, S. L. Jessen, E. Ungchusri, K. Diguette, H. R. Smith, S. M. Hasan, D. Nash, F. J. Clubb, and D. J. Maitland, "Design and verification of a shape memory polymer peripheral occlusion device," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 63, pp. 195–206, 2016. [Online]. Available: http://dx.doi.org/10.1016/j.jmbbm.2016.06.019

[103] J. N. Rodriquez, M. M. Miller, A. Boyle, J. Horn, C.-K. Yang, T. S. Wilson, J. M. Ortega, W. Small, L. Nash, H. Skoog, and D. J. Maitland, "Reticulation of low density shape memory polymer foam with an in vivo demonstration of vascular occlusion," *J Mech Behav Biomed Mater*, vol. December, no. 40, pp. 102–114, 2014.

[104] "US Food & Drug Administration: 510(k)," Silver Spring, pp. 1–7, 2019. [Online]. Available: https://www.accessdata.fda.gov/cdrh$_d ocs/pdf18/K182390.pdf$

CHAPTER

8 Appendix

8.1 Rose-questionnaire

Vraag **Heeft u ooit pijn, of andere klachten gehad zoals een drukkend, beklemmend of een zwaar gevoel op de borst (met of zonder uitstraling naar linker arm, kaak, en/of schouderbladen/rug)?**
Antwoord Nee; 0 Ja: 1

Vraag **Krijgt u die klachten ook tijdens rustig wandelen?**
Antwoord Nee: 0 Ja: 1

Vraag **Krijgt u die klachten wanneer u tegen een helling oploopt, als u moet haasten of als u een trap oploopt?**
Antwoord Nee: 0 Ja: 1

Vraag **Wat doet u als u die klachten krijgt wanneer u aan het lopen bent?**
Antwoord Doorlopen: 0 Loop langzamer of stop: 1

Vraag **Verdwijnen die klachten als u stopt met lopen?**
Antwoord Ja: 0 Nee: 1

Vraag **Als die klachten verdwijnen, hoe snel?**
Antwoord Binnen 10 minuten: 0 Na 10 minuten: 1

Indien score ≥2, dan cardioloog raadplegen voorafgaand aan het onderzoek.

Met dank aan:
Dr. M. van der Vlugt, cardioloog, Radboudumc.

Referentie:
Rose G, McCartney P, Reid DD. Self-administration of a questionnaire on chest pain and intermittent claudication. Br J Prev Social Med 1977; 31: 42.