Blood flow quantification
In the aortoiliac arteries:
from bench to bedside

Stefan Engelhard
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BLOOD FLOW QUANTIFICATION IN THE AORTOILIAC ARTERIES: FROM BENCH TO BEDSIDE

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Blood flow quantification in the aortoiliac arteries: from bench to bedside
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Chapter 1
introduction
This thesis aims to bring a new blood flow quantification technique, termed echoPIV, from the bench to the bedside of patients that suffer from aortoiliac occlusive disease. The introduction starts with an overview of the disease, including its pathophysiology and treatment options. Thereafter, echoPIV, consisting of high-frame-rate, contrast-enhanced ultrasound and particle image velocimetry, will be described in-depth. Finally, an outline of this thesis will be given.

Aortoiliac occlusive disease
Aortoiliac occlusive disease (AIOD) is a class of peripheral arterial disease (PAD) that affects the abdominal aorta and/or iliac arteries. As the disease advances, narrowing stenotic lesions or obstructions form that may compromise the supply of oxygenized blood to the lower extremities.

The prevalence of PAD increases with age, from 3-10% in people over 40 years old to about half of all people over 85 years old [1], [2]. The most common symptom of PAD, including AIOD, is intermittent claudication, or lower extremity muscle pain during exercise that is relieved after a short period of rest. However, this symptom is still only reported in 6.3% of people with PAD [2], indicating that the majority of patients is asymptomatic. As the disease progresses, it may also cause pain at rest and ultimately necrosis and tissue loss, which can lead to partial or complete limb amputation.

Pathophysiology
The pathologic mechanism that causes PAD and AIOD is atherosclerosis, a complex cellular process with several stages. In the first stage of the disease, monocytes, a type of white blood cell, attach to adhesion molecules expressed by endothelial cells of the arterial vessel wall (figure 1B). This endothelial activation can be caused by abnormalities such as hyperlipidaemia and hypertension [3]. Other risk factors that affect vascular biology are smoking and diabetes, but these mechanisms are less well understood.

After adhesion, these monocytes migrate into the intimal layer of the vessel wall, where they differentiate into macrophages and subsequently, through the uptake of lipoproteins, into foam cells (figure 1B). T-cells in the intimal layer of the vessel wall then produce cytokines that cause inflammation, forming the initial fatty streaks [4]. Both the adhesion and migration of monocytes are influenced by local hemodynamic factors, such as wall shear stress (WSS) [5]–[7], which is further discussed in chapter 2 of this thesis.
During progression of the lesion, proliferating smooth muscle cells in the intimal layer synthesize extracellular matrix molecules, such as collagen, that form the fibrous cap of the atherosclerotic lesion (figure 1C). Some of the foam cells inside the fibrous cap die, for example through apoptosis. This releases extracellular lipids that accumulate inside the lesion, resulting in a necrotic core [3]. These advanced lesions can cause a flow limiting stenosis. Alternatively, a thrombus can be formed following rupture of the fibrous cap (figure 1D) where thrombogenic material in the lipid core is exposed to the blood. This can lead to an acute occlusion of the vessel or alternatively, the thrombus can detach from the lesion causing distal embolization [3].

In patients with elevated calcium and phosphate levels in the blood (due to old age, chronic kidney disease or diabetes), hydroxyapatite crystals can also form atherosclerotic lesions, leading to calcification [8], [9]. In the coronary arteries, microcalcifications increase the risk of plaque rupture and subsequent cardiovascular events [10]. Although this relation is less well studied in peripheral arteries, it is well known that large calcified lesions can complicate or completely prevent successful endovascular treatment [8]. Furthermore, these lesions cause signal drop-out in ultrasound imaging.
Diagnosis
The primary diagnosis of PAD comes from an ankle-brachial index (ABI) measurement, or the ratio between systolic blood pressure in the ankle and the upper arm. An ABI of <0.9 confirms the official diagnosis and lower indices correlate with disease severity [11]. However, in patients with calcified lesions, the arteries in the ankles can become incompressible, leading to inaccurate high pressure readings.
Duplex ultrasound is the first-line imaging method to determine the location and severity of stenotic lesions [11], using the peak systolic velocity ratio derived from Pulse-Wave Doppler measurements. More invasive diagnostic tools such as computed tomography angiography (CTA) or magnetic resonance imaging (MRI), that require injection of contrast fluids, are often used for interventional planning. The limitations of these techniques are further discussed in chapter 2 of this thesis.

Treatment
Primary treatment of AIOD is aimed at relieving symptoms and at preventing secondary cardiovascular events, such as myocardial infarction and stroke. Treatment options include: lifestyle changes (primarily smoking cessation), medical therapy, exercise therapy and revascularization strategies. Medical therapy is aimed at decreasing the risk of cardiovascular events and includes antihypertensive, lipid-lowering and antiplatelet drugs [12]. Supervised exercise therapy is the first treatment choice in all patients with intermittent claudication. Exercise therapy can improve claudication symptoms through the formation of collateral vessels and other mechanisms, such as the reversal of endothelial and metabolic dysfunction [13].

Figure 2: X-ray images of complex stent configurations in the aortic bifurcation of an in vitro model. A: Covered kissing stents (KS). B: CERAB configuration. Adapted with permission from Groot Jebbink et al [14].
Surgical or endovascular revascularization is required when patients develop disabling symptoms that do not respond to exercise therapy, or in patients with critical limb threatening ischemia. In single stenotic lesions, plain balloon angioplasty is often performed, although additional stent placement is often indicated due to residual stenosis, recoil or dissection. Furthermore, the rate of restenosis after balloon angioplasty is 20-30% higher, compared to primary stenting, due to uninterrupted plaque growth [12]. In primary stenting, bare metal or covered stents can be used, with covered stents showing superior patency and clinical outcomes, by reducing neo-intimal hyperplasia, particularly in the more complex lesions and occlusions [15], [16].

Although international guidelines still recommend open surgery in the most advanced aortoiliac lesions [11], [17], experienced centers have shifted to an endovascular-first strategy in recent years, due to the continuous evolution of new devices and techniques [18]. Endovascular treatment has several benefits compared to open surgery, such as shorter hospital stay (4 vs. 13 days), lower complication rate (13.4% vs. 18%), decreased 30-day mortality (0.7% vs. 2.6%) and lower in-hospital costs ($13,661 vs. $17,161) [19], [20]. However, the reported short and long-term primary patency rates are still lower after endovascular treatment (86.0% vs. 94.8% after 1 year, 71.4% vs. 82.7% after 5 years) [19]. Nonetheless, re-interventions are often relatively easy to perform, resulting in secondary patency rates similar to surgery, while avoiding the risks inherent to conventional surgery [21], [22].

To treat extensive aortoiliac lesions with an endovascular approach, complex configurations of multiple stents are required, such as the kissing stent (KS) technique or Covered Endovascular Reconstruction of the Aortic Bifurcation (CERAB). In the KS technique, 2 stents are simultaneously deployed in the aortic bifurcation (figure 2A). This procedure has a primary and secondary patency rate of 63-65% and 81-82% respectively, after 5 years [23], [24]. In the CERAB technique, an aortic cuff with a funnelled shape is placed in the distal aorta in which the two iliac stents are then simultaneously placed (figure 2B). Overall medium-term results of this technique are better than observed in KS cohorts in separate studies, while more patients with extensive lesions were treated in the investigated CERAB cohorts [25]. In a recent smaller cohort, the primary and secondary patency rate after 5 years was 83% and 95%, respectively [26].

Several geometric factors, like radial mismatch between the diameter of the stents and the aorta, have an adverse effect on the patency results of these complex stent configurations [14], [27]. These geometric factors also induce changes to local blood flow, that could explain this negative effect, as further discussed in chapter 2 of this thesis. Another risk factor for stent failure is poor distal run-off, caused by stenotic lesions distal to the stent or diseased microvasculature [28]–[30]. The effect of a distal stenotic lesion on the CERAB configuration is investigated in chapter 3 of this thesis. These findings show the need for accurate flow quantification in patients with aorto-iliac stents.
High-frame-rate, contrast-enhanced ultrasound velocimetry

One of the techniques that can be used for blood flow quantification, is echoPIV (a detailed review of alternative techniques can be found in chapter 2 of this thesis). The echoPIV technique consists of acquiring ultrasound images at high frame rates after injecting a microbubble contrast agent, i.e. high-frame-rate, contrast-enhanced ultrasound, or HFR-CEUS. After post-processing, the acquired images are used for particle image velocimetry (PIV) analysis, where the displacement of the microbubbles within these images is calculated, to represent the local blood flow (figure 3).

**Figure 3. Overview of the echoPIV method. This method consists of injecting a microbubble contrast agent (A), capturing images at very high frame rates with plane wave ultrasound (B) and then calculating the displacement of the microbubbles in these images with PIV (C).**

*Plane wave ultrasound*

In conventional ultrasound imaging, multiple focused ultrasound beams are transmitted and the reflection of each beam is used to construct one line of the image. The frame rate, typically around 50 Hz, is limited by the number of lines per image and is usually insufficient for 2-dimensional quantification of the physiologic blood flow velocities in large arteries (around 1 meter/second).

In plane wave US, all elements in the transducer are activated simultaneously, transmitting an ultrasound wave that covers the entire region of interest. A full image is reconstructed for each transmit, allowing framerates up to 10 kHz at an average arterial depth [31]. To increase image quality, several tilted plane waves can be transmitted and coherently summed to construct an image, with similar quality to conventional imaging but at framerates around 10-100 times higher [31].

To quantify blood flow, scattered US signals from the blood are needed. In superficial vessels such as the carotid artery, it is possible to quantify blood flow by detecting the backscattered US signal from red blood cells and calculating their displacement [32]. However, in deeper vessels such as the abdominal aorta, ultrasound contrast agents are often required to enhance US backscattering from the blood pool.
**Contrast Microbubbles**

Several classes of ultrasound contrast agents exist, such as solid or liquid nanoparticles, or liquid filled liposomes [33]. In medical applications, gas filled microbubbles with a stabilized phospholipid shell are almost exclusively used as a contrast agent. These microbubbles are 1-10 μm in diameter and can pass through the pulmonary capillaries, but do not diffuse out of the circulation, making them very suitable for vascular imaging [34]. Clinically approved microbubbles show an excellent safety profile, compared to radiologic or MRI contrast agents [35]. The acoustic impedance mismatch between microbubbles and blood makes them highly echogenic. Furthermore, nonlinear resonant behavior creates harmonic signals that can be separated from the mostly linear tissue signal with specialized contrast imaging sequences, such as power modulation, pulse inversion or a combination of both [36]–[38].

**Singular Value Decomposition**

Instead of utilizing harmonic signals from the microbubbles for clear visualization, they can also be separated from tissue signal by analyzing their spatiotemporal characteristics, by performing singular value decomposition (SVD) on the obtained image data. The first step in SVD is constructing a 2-dimensional “Casorati” matrix from the 3-dimensional image stack, where each column represents a single image and each row represents the same pixel over time. This matrix (S) is then decomposed as follows:

\[ S = U \Delta V^* \]

Where U and V are orthonormal matrices, whose columns represent the spatial and temporal singular vectors of S, respectively [39]. \( \Delta \) is a diagonal matrix consisting of singular values. Tissue signal, with a higher spatial and temporal coherence, is mostly present in the first singular vectors of U and V. A filtering matrix (\( I^f \)) can be used that removes the contribution of the corresponding singular values in \( \Delta \), leading to a filtered Casorati matrix (\( S^f \)).

\[ S^f = U \Delta I^f V^* \]

This filtered matrix can then be transformed back into an ultrasound image stack, from which the tissue signal is ideally removed. The threshold for the truncation of the diagonal matrix \( \Delta \) can be set manually, but several automatic threshold algorithms also exist [40]. These algorithms can be based on the magnitude of the singular values in \( \Delta \), i.e. their coherence energy (chapter 4 & 5), or on the characteristics of the temporal and spatial singular vectors in U and V (chapter 6 & 7). The resulting SVD filtered images can be used for blood flow analysis, for example by particle image velocimetry (PIV).
**Particle Image Velocimetry**

In PIV, the displacement of groups of particles is calculated between two consecutive images, taken at different times (∆t), resulting in a local velocity vector [41]. This displacement can be calculated by performing a cross-correlation of sub-regions (or kernels) within the images, either directly using a statistical approach, or in the Fourier domain. The peak in the resulting correlation matrix represents the most probable displacement of particles in the kernel, from which the local flow velocity vector is then calculated (figure 4).

In cases of high velocity, correlation between the kernel pairs decreases, as some of the particles move outside the kernel in the time between the images. This problem can be overcome by utilizing multiple iterations, or “multi-pass” methods, using a larger kernel size in the first pass and displacing or shifting the kernels in subsequent passes. Another problem that can occur is shear, or high velocity gradients, that cause particles within a kernel to move at different speeds, subsequently reducing correlation values. This can be tackled by not only shifting, but also deforming kernels in subsequent passes [42].

In most scientific fields, PIV images are acquired with a high-speed camera, in a transparent model containing a fluid with fluorescent particles, illuminated with a laser [43]. This technique, also referred to as “laserPIV”, is used in chapter 3 of this thesis to investigate the re-occlusion of a stent, a common clinical problem in vascular surgery. With advanced PIV techniques such as stereoscopic PIV [44] or tomographic PIV [45], it’s also possible to obtain high-resolution 3-dimensional flow fields from complex flow set-ups, in controlled conditions. However, laserPIV cannot be used inside the human body. Fortunately, PIV analysis can also performed on HFR-CEUS images, which is called echoPIV. In chapters 4-7, the feasibility and clinical application of echoPIV is investigated, in human volunteers and in patients with aortoiliac disease.

![Figure 3: overview of the echoPIV method. Each image is divided into subregions (kernels), of which the cross-correlation with a subsequent image is calculated. The peak in the correlation map is then used to estimate the regional velocity.](image-url)
Thesis outline

The main goal of this thesis is to investigate the feasibility and clinical application of echoPIV, in order to move this technique forward from the bench to the patient’s bedside, thereby improving clinical care. Within this thesis, studies were carried out in healthy volunteers and patients with aortoiliac disease, with and without stents, to achieve this goal.

In chapter 2, current diagnostic techniques for aortoiliac occlusive disease are discussed, including their capacities and limitations. Here, it becomes clear that there is a need for improved methods to estimate lesion severity or predict disease progression and stent patency, based on local blood flow. This is followed by an extensive overview of novel blood flow quantification techniques that can be used to meet this demand.

With widespread availability of these in vivo techniques still lacking, patient-specific evaluation of local blood flow patterns in a clinical setting is currently not possible. However, valuable general insights into blood flow related issues can still be obtained in vitro. In chapter 3, the occlusion or re-stenosis of a stent due to poor distal outflow, which is a common clinical problem, is investigated using laserPIV in a transparent model with a simplified anatomy.

In chapters 4 and 5, the feasibility of echoPIV in healthy volunteers is investigated, including a parameter study into different acquisition and processing settings (chapter 4) and a comparison of the acquired velocity data with 4D flow MRI (chapter 5).

The feasibility and clinical application of echoPIV in patients with aortoiliac disease, with and without stents, is investigated in chapters 6 and 7. In patients without a stent (chapter 6), new parameters to quantify disturbed flow are investigated. In patients with a stent (chapter 7), the robustness of flow visualization inside these stents is investigated.

In chapter 8, the main results of this thesis are summarized and future perspectives are discussed.
Blood flow quantification in peripheral arterial disease: emerging diagnostic techniques in vascular surgery

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Abstract

The assessment of local blood flow patterns in patients with peripheral arterial disease is clinically relevant, since these patterns are related to atherosclerotic disease progression and loss of patency in stents placed in peripheral arteries, through mechanisms such as recirculating flow and low wall shear stress (WSS). However, imaging of vascular flow in these patients is technically challenging due to the often complex flow patterns that occur near atherosclerotic lesions. While several flow quantification techniques have been developed that could improve the outcomes of vascular interventions, accurate 2D or 3D blood flow quantification is not yet used in clinical practice.

This article provides an overview of several important topics that concern the quantification of blood flow in patients with peripheral arterial disease. The hemodynamic mechanisms involved in the development of atherosclerosis and the current clinical practice in the diagnosis of this disease are discussed, showing the unmet need for improved and validated flow quantification techniques in daily clinical practice. This discussion is followed by a showcase of state-of-the-art blood flow quantification techniques and how these could be used before, during and after treatment of stenotic lesions to improve clinical outcomes. These techniques include novel ultrasound-based methods, Phase-Contrast Magnetic Resonance Imaging (PC-MRI) and Computational Fluid Dynamics (CFD). The last section discusses future perspectives, with advanced (hybrid) imaging techniques and artificial intelligence, including the implementation of these techniques in clinical practice.

Introduction

Peripheral artery disease (PAD) is defined as partial or complete stenosis of peripheral arteries, usually caused by atherosclerosis. PAD is associated with risk factors similar to those for atherosclerotic coronary artery and cerebrovascular diseases, and is very common among the elderly. Over the past decade, there has been a 24% increase in the prevalence of PAD worldwide and this has had a global impact on health care economics [46]. PAD has a prevalence of 15-20% in persons aged over 70 years and 6-8% of them suffer from intermittent claudication, which is defined as leg pain brought about by walking [46]. Intermittent claudication may impact the patient’s quality of life and is usually treated with walking exercise training, but revascularization is indicated in cases of persistent disabling complaints. Furthermore, with disease progression, critical limb ischemia may develop, which carries a significant risk of lower limb amputation.
**Hemodynamic mechanisms of vascular diseases**

The pathologic mechanism that causes PAD is mostly atherosclerosis. In addition to the well-known classic risk factors, including nicotine abuse, hypertension, dyslipidemia and diabetes mellitus, blood flow-related biological mechanisms can influence the formation and development of atherosclerotic plaques in the human vasculature. For example, low or oscillatory wall shear stress (WSS) causes a disturbed pattern of endothelial cell growth [47]–[49], with the consequence that endothelial cells are no longer aligned in the direction of the blood flow (figure 1). This allows inflammatory cells to pass through the endothelium more freely. Furthermore, disturbed mecano-transduction by glycocalyx molecules on endothelial cell membranes, due to changes in blood flow, causes an increased adherence and migration of inflammatory cells through the endothelium [50], [51]. This, in turn, further strengthens the inflammatory response and subsequent atherosclerotic process.

Several studies have suggested that derived flow parameters such as WSS and oscillatory shear index (OSI) strongly influence the development and progression of atherosclerotic lesions [48]. More specifically, atherosclerotic disease is predominantly found in regions with low WSS and high OSI [52], [53]. In arteries such as the coronary and carotid arteries, these parameters have been shown to predict plaque vulnerability, leading to poor clinical outcomes [54].

![Figure 1. Endothelial cells exposed to different levels of shear stress. In physiologic shear stress conditions (A), endothelial cells align in the direction of blood flow. In abnormal conditions, such as low shear stress (B), oscillating flow (C) or stasis (D), the endothelial cells show abnormal growth patterns that make the vessel wall more prone to atherosclerosis. Reprinted with permission from Conway et al [48].](image_url)
Local blood flow patterns may also define treatment success for atherosclerotic lesions. For example, several factors related to the design of bare metal stents, the stent configuration and procedural aspects can influence the local geometry and, subsequently, local blood flow patterns [55]. These blood flow disturbances often cause areas with low WSS, thereby modulating the inflammatory response to endothelial injury caused by stent placement, leading to neointimal hyperplasia [55].

In atherosclerotic lesions of the aortic bifurcation, which are often treated with a kissing stent configuration, several geometric factors have been identified that influence the patency rate of these stents, such as stent crossing and radial mismatch [23], [56], [57]. An \textit{in vitro} study that compared several aorto-iliac stent configurations with different geometries showed that blood flow recirculation takes place in the inflow tract of the kissing stent configuration with covered stents and at the native bifurcation in the kissing stent configuration with bare metal stents [58]. Clinically, these areas often manifest as sites for restenosis. Moreover, hemodynamic alterations, related to geometrical mismatch, may lead to the presence of immature mesenchymal tissue, intimal hyperplasia, and organizing thrombus, not only in the space created inferior to the vertex of the opposing stents, but also within the free-floating portions of the stents [59].

Poor distal run-off has also been identified as an important risk factor for stent failure [28]–[30]. Another \textit{in vitro} study, which compared models with and without a complex stent configuration and an identical stenotic lesion in the outflow tract, showed that the decrease in WSS caused by the stenotic lesion was greater in the stented model [60]. This may be a mechanism behind stent failure in segments with poor distal run-off.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{vector_doppler}
\caption{Vector Doppler uses multiple US beams at different angles to obtain the Doppler shift and corresponding flow velocity in two different directions (green and light blue arrows). This is then converted into the actual 2-dimensional velocity vector (dark blue arrow). Reprinted with permission from Jensen et al [61].}
\end{figure}
Although *in vitro* studies such as these provide useful general knowledge about hemodynamics and possible failure mechanisms of stents, accurate flow quantification *in vivo* is needed to develop clinically applicable techniques that can be used in patient-specific situations.

**Diagnostics in vascular surgery**

In symptomatic patients with PAD, computed tomography angiography (CTA) scans and duplex ultrasound (US) are routinely used to obtain anatomic and flow information, respectively.

The physics of fluids dictate that the pressure or energy loss of a stenosis is primarily caused by the lumen area expansion, which forces blood into complex recirculating motions as it exits the stenosis [62]. The maximal diameter or area reduction caused by a stenosis can thus be viewed as the most important parameter of hemodynamic stenosis severity. This can be assessed on CTA, with the radiation burden and overestimation of the stenosis severity in case of calcification artefacts as potential drawbacks.

The US-derived peak systolic velocity (PSV) ratio provides an estimate of the hemodynamic severity of the stenosis. It is defined as the ratio of the intra-stenotic PSV and the pre- or post-stenotic PSV and assumes that the blood flow velocity is inversely related to the change in area caused by a stenotic lesion. The blood flow velocities in duplex US are estimated with pulsed wave Doppler, which depends on the angle between the flow and the US beam. These estimates are inaccurate in areas where blood flow is almost perpendicular to the US beam [63]. Furthermore, the flow direction and corresponding angle with the US beam cannot always be reliably determined by the operator in complex flows near bifurcations and stenotic lesions. This leads to inaccurate velocity calculation, contributing to errors in the estimation of lesion severity [64], [65].

![Figure 3. The Transverse Oscillations technique uses a specialized ultrasound beam with an extra oscillation in the lateral direction (right side), as opposed to a regular ultrasound beam that only oscillates in the axial direction (left side). This can be used to obtain velocity information in 2 directions. Reprinted with permission from Udesen et al [66].](image-url)
Duplex US has a moderate sensitivity and specificity for the detection of significant lesions (74-83% and 67-93%, respectively) compared to invasive pressure-gradient measurements [67]–[69]. This discrepancy is clear from a physical perspective, as pressure loss depends not only on the ratio of flow velocities caused by the area reduction, but also on the size of the recirculation zone and the dissipation of energy in turbulent flow disturbances, which have more complex relationships with the stenosis geometry [62]. Therefore, the PSV ratio alone is not accurate enough to evaluate the significance of arterial stenosis, especially in borderline cases. Furthermore, stenotic lesions exhibit various anatomical morphologies with possibly a similar pressure loss, but with entirely different blood flow patterns, leading to different rates of disease progression and subsequent clinical outcomes.

Thus, there is a clear need for improved blood flow quantification to improve the assessment of stenosis severity, the prediction of disease progression and the outcomes of (endo)vascular procedures. This article showcases current state-of-the-art techniques, including US, PC-MRI and CFD, followed by future perspectives in advanced (hybrid) imaging techniques and artificial intelligence. Special attention is given to the use of these innovative technologies before, during and after (endovascular) treatment of narrowed arteries.

![Figure 4. Blood flow velocity estimated with Transverse Oscillations in 2 patients with a stenosis in the superficial femoral artery. Direction and velocity are represented by the color wheel, lesions are marked with an asterisk. A: Flow in this patient is unidirectional. B: Complex, multi-directional post-stenotic flow. This flow complexity can be used to determine lesion severity. Reprinted with permission from Hansen et al [70].](image-url)
Novel blood flow quantification techniques

*Flow quantification with ultrasound*

The use of ultrasound allows for easy, real-time imaging at relatively low cost, with portable devices that allow bedside diagnosis. US examinations have been a common diagnostic tool for decades in many clinical areas, such as cardiology, orthopedics and gastroenterology. Blood flow velocity can be estimated with Doppler US, although it has many limitations.

Several techniques are currently under development to enable 2-dimensional quantification of blood flow, thereby overcoming many limitations of conventional Doppler US. These techniques either use advanced methods to use the Doppler shift in more than one direction, or rely on blood speckle tracking.

Vector Doppler uses two US beams to obtain the Doppler shift and corresponding flow velocity in two separate directions (figure 2). These two velocity components are then used to calculate a 2-dimensional velocity field [61]. Vector Doppler is not angle-dependent, is less operator-dependent and has been shown to be more accurate than conventional Doppler approaches,[71] including with complex blood flows.[72]

Another Doppler-based technique is called Transverse Oscillation (TO) [73]. In this technique, only one US beam is used. By adding a transverse wave to this beam (figure 3), both the axial and transverse velocity components can be extracted [66], [73]. TO has been used in the femoral artery to improve the classification of stenosis severity (figure 4) and is currently available on select clinical US machines [70].

![Figure 5. In Vector flow mapping (VFM), automatic segmentation is performed on conventional Doppler US data to obtain vessel wall motion (left). Blood flow regularization is then performed, assuming flow continuity and in-plane flow, to obtain the cross-beam (angular) velocity component (middle). The angular component is used together with the original (radial) velocity component to construct 2-dimensional velocity vectors (right). Reprinted with permission from Assi et al [74].](image-url)
Vector flow mapping (VFM) uses conventional Doppler US data to obtain the cross-beam (angular) velocity component and subsequently the 2D velocity field [75]–[77]. Automatic segmentation is performed to detect wall motion, which is used together with a flow continuity equation and in-plane flow assumption to calculate the angular velocity component (figure 5) [74]. VFM is commercially available and has been used in many clinical studies to study cardiac flow [78]–[82], but has not yet been applied to peripheral arteries.

Non-Doppler-based techniques also exist and of these, speckle tracking has been most widely investigated. In this method, the speckle pattern resulting from US scattering by red blood cells is tracked over time to yield 2-dimensional blood flow information [83]. Speckle tracking has been used in a range of applications, including the visualization of valvular helical flow in the great saphenous vein [84] and complex flow in the carotid artery [32]. This technique is also available on clinical US machines [85].

Although the techniques mentioned above are relatively inexpensive, (in part) commercially available, easy to use and more accurate than conventional duplex US, most of them have a limited depth [86]. In Vector Doppler and TO, a wide transducer aperture is required relative to the imaging depth to allow calculation of the Doppler shift in two directions. Speckle tracking suffers from a low signal-to-noise ratio, which makes data processing for deeper tissues challenging. Still, the widespread application of these techniques in clinical practice is currently possible, and could improve the estimation of lesion severity [70]. Novel flow parameters can also be used to quantify flow-disturbing factors such as vector concentration or vector complexity, a measure of the multi-directionality of the flow field [32], [70], [72]. This could significantly improve the diagnosis and monitoring of patients with PAD.

![Figure 6. In ultrasound velocimetry (echoPIV), High-Frame-Rate, Contrast-Enhanced Ultrasound (HFR-CEUS) images are used for particle image velocimetry (PIV) analysis, by cross-correlation of matching sub-regions. The displacement of particles in the image sub region is estimated from the peak in the correlation map, resulting in a 2-dimensional vector.](image)
Ultrasound particle image velocimetry (echoPIV)

A relatively new technique that can overcome the depth limitation of other US-based methods is contrast-enhanced US particle image velocimetry (echoPIV). With this technique, contrast microbubbles are injected and US images are subsequently acquired at very high frame rates using “plane wave” US beams [87]. In plane wave US, each image can be constructed from a single transmitted wave that covers the entire field of view, as opposed to conventional B-mode imaging where >100 focused US beams are necessary to form an image. Compounding of different transmit angles provides images with a quality similar to that in conventional US imaging, while thousands of images per second can still be obtained. These images are then used in particle image velocimetry (PIV) analysis to obtain two-dimensional velocity vector fields of blood flow [88]. PIV consists of calculating the cross-correlation of multiple subregions between consecutive image pairs (figure 6), similar to speckle tracking in non-contrast US images. Since the maximum velocities that can be found are limited by the frame-rate, conventional B-mode US cannot be used for this technique.

![Figure 7. Blood flow velocities in 2 patients with a stent in the external iliac artery (EIA), represented by streamlines. The vessel wall (white) and stent (red) were drawn manually. A: Flow quantification suffers from a calcification artefact proximal to the stent (red arrow), but undisturbed flow can clearly be observed in both the inflow and outflow tracts. B: A recirculation zone occurs near the distal edge of the stent (yellow arrow), which could lead to restenosis.](image-url)
In the left ventricle, echoPIV has been used to quantify two-dimensional blood flow velocity and derived parameters such as vortex strength, which is related to poor mechanical performance and clinical outcome [89]. In the carotid artery, accurate calculation of WSS was shown to be feasible with echoPIV [90]. More recently, the feasibility of echoPIV for blood flow quantification in the abdominal aorta was demonstrated in a group of healthy volunteers [91]. The flow parameters showed very good agreement with MRI data obtained from the same subjects. In an ongoing study, the feasibility of this technique is being tested in a large cohort of patients with aorto-iliac occlusive disease, including a subgroup that was treated with stents. In this study, recirculation zones were found in several patients near the inflow or outflow tract of the stent (figure 7). The prognostic value of these findings is currently under review.

The echoPIV technique can be used in deep lying vessels and the heart, making it a promising tool for blood flow quantification around stenotic lesions, such as in the abdominal aorta [86]. However, contrast injections are needed, making it unsuitable for widespread use during the early diagnosis of aorto-iliac disease. Furthermore, data processing is currently performed offline, unlike in Doppler-based methods, so direct feedback during measurements is not available.

In the future, however, the prediction of disease progression and long-term stent patency of vascular stents with echoPIV could be a great improvement in the treatment of patients with vascular disease. Microbubble contrast agents have an excellent safety profile compared to iodinated contrast agents used in CTA scans [35]. Several studies have already suggested that contrast US scans could replace CTA scans for the detection of endoleaks in follow-up after endovascular aneurysm repair [92]–[94], thereby drastically reducing radiation exposure in this patient group. Similarly, echoPIV (or other US-based techniques) could replace CTA scans in the evaluation and treatment planning of PAD. More specifically, the availability of a more accurate estimation of lesion severity, based on the pressure gradient derived from non-invasive flow quantification, could lead to a better-informed choice of which lesion to treat first, instead of the standard protocol of opting for the most proximal lesion. During the procedure, real-time flow quantification with US could be used to evaluate the placement of a stent or bypass graft. Possible flow disturbances could then be immediately addressed, improving the outcome of the procedure.

**Phase-contrast MRI**

Cine three-directional phase contrast magnetic resonance imaging (PC-MRI) is a valuable angiographic technique for visualizing blood flow [95]. It provides 3-dimensional flow information in a large field of view, without the need for contrast agents. The magnitude of blood flow velocity (i.e., speed and direction) can be quantified from the phase signal of the data (i.e., 4D Flow MRI).
Phase differences are created with gradient echo acquisitions by spins moving along a bipolar gradient in the magnetic field. This bipolar gradient causes a subsequently positive and negative phase difference that cancels out for non-moving particles, while a phase difference remains for moving particles that is proportional to their velocity [96]. To correct for inhomogeneities in the magnetic field, another bipolar gradient with opposite polarity is applied. These phase images are generated for three velocity directions separately and then combined to provide 3-dimensional velocity quantification, usually reconstructed as 20-40 frames per heartbeat (figure 8).

Figure 8. 4D flow MRI is ECG-gated and uses a bipolar encoding gradient for each of the 3 directions separately. In this way, velocities in three directions are encoded separately as phase differences over multiple (100+) heart cycles. After processing, this provides a 3-dimensional visualization of blood flow that can be used for quantitative analysis, by calculating parameters such as WSS. Reprinted with permission from Markl et al [96].
Several parameters can be calculated from the 3-dimensional flow field, such as (turbulent) kinetic energy, pressure loss and WSS [97], although the accuracy of these parameters is limited by the spatial and temporal resolution of the scan [98]. PC-MRI is most widely investigated for applications in the heart and thoracic aorta [99]. However, several authors have described applications in the abdomen, such as the portal vein [100] and mesenteric artery, to investigate postprandial blood flow in patients with suspected chronic mesenteric ischemia [101]. Complex helical flow distal to a moderate stenosis in the common iliac artery has also been successfully quantified (figure 9) [102]. Although PC-MRI offers accurate 3-dimensional flow visualizations in large volumes of interest and is widely available, it is relatively expensive and time-consuming (with scan times of 10-20 minutes), currently making it less suitable for widespread clinical use in patients with PAD, especially in the early phase of the disease. The sequences used are unfit for real-time imaging, making it necessary to divide the acquisitions over many cardiac cycles and then construct an average heartbeat, which is also known as interleaved sampling. Newer methods can correct for respiratory motion to reduce scan times to around 4 minutes and variable, turbulent flows can also be resolved with this technique, although this still does not provide instantaneous flow velocities during a single heartbeat [103]. Finally, metal stents cause disturbances in the magnetic field that lead to artefacts in the MRI images, making it very difficult to quantify blood flow close to or inside stents [104], [105].

Figure 9. Example of PC-MRI in the aorto-iliac region of a patient with PAD. A: Coronal MRA slice of the aorta and left iliac arteries. B: color-coded 3D streamline representation of blood flow velocities, showing vortical flow in the dilated common iliac artery (yellow box). These scans can be used to guide pre-operative decision making. Reprinted with permission from Frydrychowicz et al [102].
Nevertheless, PC-MRI has been proven to be useful in many research areas and could be used to gain further insights into the disease mechanisms behind PAD. In a clinical setting, it could be used in combination with other techniques, or as a reference standard. The greatest strength of PC-MRI is its ability to scan a large area at once. Newer methods that involve machine learning can improve blood-tissue contrast [106] and could replace conventional CT or MR angiography scans. If such a scan is indicated in a patient with PAD, an MRI flow sequence could be easily added to the clinical workflow to investigate flow abnormalities in the entire peripheral vascular tree.

**Computational Fluid Dynamics**

Computational modeling for vascular surgery introduces a variety of promising capabilities for diagnosis and treatment planning in patients with PAD. From both clinical and methodological perspectives, it is convenient to classify these computational approaches into fluid mechanical models of blood flow that neglect wall motion, biomechanical models of vessel wall motion, and combined models of blood and wall motion that include fluid-structure interactions. The clinical pathology determines the most suitable approach.

For the diagnosis of a stenosis or the prediction of atherosclerotic disease progression, computational fluid dynamics (CFD) models (with a rigid vessel wall) can provide relevant insights, as the introduction of compliant vessel walls typically has a limited effect on the outcome of the simulation [107]. To investigate wall rupture or wall-stent interaction, a biomechanical approach is required. Fluid structure interaction combines these two approaches and helps in understanding the interplay between the flow field and vessel wall stresses, which is important in cases of, for example, plaque rupture.

![Figure 10. A clinical CFD simulation can be subdivided in three stages: (1) Obtaining anatomic and functional patient imaging data, (2) building a patient model consisting of a discretized geometry and inflow and outflow boundary conditions, and (3) computing the 3D flow velocity field in a patient and extracting hemodynamic parameters of interest.](image-url)
For current clinical applications, CFD modeling is most commonly used and provides estimates of hemodynamically relevant quantities like pressure, blood flow velocity and WSS that can be related to the disease burden or surgical outcome for a particular patient. Construction of the CFD model requires faithful segmentation of the vessel wall from medical images, and the flow rate of blood at the inflow and outflow sections are needed as “boundary conditions” (figure 10). The number of arteries included in a CFD model is therefore bound by the availability of anatomic imaging data, which at present limits its clinical application to vessels larger than ~1 mm. In practice, blood flow is often only simulated in a single major artery or a major bifurcation.

Lumen segmentation for CFD can be based on 3D anatomic imaging modalities such as CT, MRI or rotational angiography. The boundary conditions can be estimated from population averages, but are preferentially derived from flow measurement techniques. All flow quantification measurement techniques mentioned in the previous sections can be used, so choosing one is a practical trade-off between the cost and availability of measurement techniques and the required accuracy of the CFD outcome. CFD computations use these two model inputs to compute the velocity, pressure and shear stresses of blood in the 3D-geometry over one or more cardiac cycles. This provides a large 4D-dataset from which pressure gradients over a stenosis, areas of flow recirculation and WSS can be derived in every location.

However, it is important to realize that the accuracy of these CFD outcomes is directly linked to the accuracy of the lumen segmentation and the flow measurements used to set the inflow and outflow rates. This may lead to unquantifiable sources of errors in the CFD solution, as was shown in a recent study with CFD in intracranial aneurysms, where the results from 26 teams across the globe varied considerably due to differences in segmentation [108]. A specific CFD algorithm, including the imaging modalities and segmentation methods used, must therefore be validated against hemodynamic measurements or directly to clinical outcomes in patients.

**Figure 11.** Blood flow velocities during systole, calculated with CFD and represented with 3-dimensional streamlines, in a patient with a stent in the superficial femoral artery (top right) and a 50% stenosis in the distal common femoral artery (CFA). The stenosis causes increased blood flow velocities in the CFA (red streamlines) and complex flow in the femoral bifurcation, possibly influencing the long-term patency of the stent.
The accuracy of CFD techniques will continue to evolve with improvements in imaging resolution and novel flow-imaging techniques. The hemodynamic parameters obtained with CFD in patients with PAD can be used in various ways to improve their diagnosis, treatment and follow-up. For diagnosing the severity of stenosis, the computed pressure gradient can be used, which could yield more accurate results than the PSV ratio obtained with duplex US. This is akin to the commercial application of HeartFlow™ (Heartflow, Redwood City, CA) for coronary artery disease [109], for which the application of CFD has been validated in thousands of cases against invasive pressure measurements [107].

Second, the application of CFD for large cohorts of stented patients may reveal flow-related causes of stent failure, like the presence of a recirculation zone with low WSS in the stent (graft). An example of CFD used for investigating stent failure is shown in figure 11. If flow-related causes of stent failure are found in this matter, CFD (or other quantification techniques) could become a very useful tool for individualizing treatment planning and follow-up. Before treatment, different stent configurations could be tested in a virtual patient model to select the optimal treatment strategy based on its flow characteristics. Subsequently, the intensity of a patient’s follow-up could be linked to a flow-based risk score.

Future perspectives

The techniques described in this article have a wide range of possibilities and limitations, and the stage of commercial availability varies greatly (Table I). Additional studies are needed to investigate which techniques are best suited for specific clinical applications. Further development of these techniques should help to overcome current limitations, paving the way for implementation in clinical practice. Promising future developments are discussed in this section.

3D echoPIV / vector flow imaging

One of the drawbacks of US-based flow quantification, compared to MRI and CFD, is that current techniques rely on a single image plane that captures only part of the 3-dimensional flow field. Another problem is that the imaged blood vessel can be partly outside of the imaging plane. This is especially relevant for patients with atherosclerosis, who often have elongated arteries. If the center plane of the artery is correctly imaged, out-of-plane blood flow velocities can still occur, due to the complex flow patterns in diseased vessels. 3-Dimensional US techniques are needed to capture these out-of-plane velocity components. While considerable effort is being devoted to the development of this technique, most applications are still in the in vitro testing phase [110], [111].
Artificial Intelligence

Artificial Intelligence, especially machine learning using neural networks (i.e., deep learning), is advancing rapidly in many research areas inside and outside healthcare. This is also the case in medical imaging, and flow quantification in particular.

In PC-MRI, machine learning algorithms can be used to increase the spatio-temporal resolution of the velocity fields and reduce noise [112], or to emulate the use of contrast agents [106]. Several parts of the US imaging chain can also be improved by using machine learning algorithms [113]. For example, so-called “physics-informed” neural networks can be trained to perform beamforming of the US images. This enhances image quality, while drastically reducing the computation time [114]. When further developed, this could allow for the use of high-frame-rate US acquisition schemes on clinically available hardware.

Automatic image segmentation can also be performed using deep learning algorithms, which has already been proven to be highly accurate for calculation of the ejection fraction in echocardiography [115]. Another study improved automatic segmentation speed and showed good agreement with manual segmentation in PC-MRI scans of the aorta [116]. These approaches could improve localization of the vessel wall and the subsequent derivation of flow parameters such as WSS. Physics-informed neural networks can also be used in PC-MRI to provide consistent estimates of velocity, blood pressure and other derived flow parameters [117]. These neural networks are constrained by fluid dynamics models, which regularize the velocity field to be physically consistent.

CFD can also be complemented by artificial intelligence-driven image segmentation algorithms, as the accuracy of the flow model depends heavily on this segmentation. Furthermore, image segmentation is the most labor-intensive step in current CFD algorithms, enhancing the need for automation to speed up this process. Alternatively, a neural network can be trained with a large set of reference CFD simulations of PAD patients to predict blood flow behavior, which can substantially decrease the computational time needed, possibly to the point of nearly real-time predictions [118]. These developments can further enhance the applicability of CFD in daily clinical practice.

CFD combined with advanced imaging techniques

As mentioned in this article, the accuracy of CFD is inherently limited by the accuracy of the anatomic and flow imaging modalities used in constructing the lumen segmentation and boundary conditions of the patient model. For the computation of a pressure gradient in large arteries, CT and MR-angiography have sufficient resolution for clinical CFD application [119]. In stent grafts, the flow patterns for the majority of the arterial lumen can be accurately obtained by CT or MR-based CF. However, the intricate flow patterns near the struts of bare metal stents in particular may not be reliably captured by CT or MR-based CFD, due to their limited resolution and susceptibility to metal artefacts.
In such cases, high-resolution intravascular imaging techniques like intravascular ultrasound (IVUS) and optical coherence tomography (OCT) could be used to provide refined segmentations of a stented arterial lumen [120]. Intravascular OCT, in particular, can yield *in vivo* images that rival histopathologic coupes, and has already been used to provide detailed segmentations of stented coronary arteries for high-fidelity CFD simulations [121]. These high-resolution anatomic images may be unnecessary for well-apposed endografts, which have a reasonably smooth lumen-facing boundary that can be well-approximated with CT. However, OCT may be essential for accurate calculation of the near-wall flows of bare metal stents, to predict intimal hyperplasia and subsequent in-stent restenosis.

Next to anatomic imaging, CFD simulations will receive instrumental benefits from the improved flow quantification techniques addressed in this article. Duplex US has moderate accuracy for measuring the flow rate in arteries, which sensitively influences CFD simulations. Accurate quantification of flow rates in the inlet and outlet boundaries of CFD models by PC-MRI, or US-based techniques such as echoPIV, can therefore improve the accuracy of the simulation. Alternatively, hybrid approaches are possible where CFD simulations are used to enhance the spatial and temporal resolution of 3D flow quantification techniques and to regularize the blood flow [122].

**Virtual stenting with CFD**

An exceptionally attractive application of the digital patient models that are produced for CFD is the modification of patient models to examine the hemodynamic consequences of treatment. In the long term, a virtual surgery software platform can be envisioned, in which different surgical and endovascular treatment strategies can be tested and evaluated for optimal hemodynamic outcomes on a case-to-case basis [123]. With current techniques, segmentation and CFD simulation may be performed within a day in a well-optimized processing pipeline, which makes CFD-based treatment planning possible if it is properly integrated in the clinical workflow. Advancements in supercomputing and CFD codes can be complemented by the development of less complex models that can compute blood flow parameters much faster than the current state-of-the-art [124], [125]. This could enable live individualized treatment planning during endovascular surgery, in which the hemodynamic effects of angioplasty or various stenting strategies could be simulated to design the optimal treatment strategy (figure 12).

The incorporation of a stent into this “virtual treatment” poses additional challenges, since stent deployment in a patient’s anatomy must be simulated and the stent influences the flow at a scale typically not represented by a simple, reduced order model. However, the mechanical simulation of how complex endovascular devices conform to a patient’s anatomy is slowly finding its way into clinical workflows [126]. When used together with subsequent CFD simulations, this allows for a patient-specific hemodynamic evaluation
of device performance. Numerous examples of virtual stenting have been reported for bare metal stents, typically using commercial structural solvers [127]–[130]. For covered stent grafts, the mechanical linking of struts through the graft material, as well as graft contact with the wall, becomes exceedingly difficult to simulate, although some success has recently been reported in case studies of tortuous aneurysms [131].

![Figure 12. An example of virtual balloon angioplasty in the common femoral artery. A: Computed flow in the femoral arteries. B: Original patient geometry, obtained with CTA, showing 2 stenotic lesions in the common femoral artery (dashed line rectangles). C: Angioplasty of the original geometry (red) with a virtual balloon (yellow). D: Virtually treated patient geometry. E: Computed blood flow in the virtually treated geometry, showing less flow acceleration in the stenotic lesions and less disturbed flow around the femoral bifurcation.](image)

**Implementation into clinical practice: personalized medicine**

Although the techniques reviewed in this article have very promising applications for vascular surgery, for most of them, their true added value has not yet been proven. Large cohort studies in patients with PAD are needed to investigate if the studied flow patterns can influence the development and progression of atherosclerotic lesions, or the patency of endovascular stents. Nonetheless, the visualization of blood flow velocities in individual patients could induce a paradigm shift in vascular surgery. Graft and stent sizing have historically been based on anatomic factors and hemodynamic intuition about which geometry creates orderly blood flow. With the advent of flow quantification in patients, these intuitions will be challenged; and while many will likely be supported, others will be disproved. Combined with research into the link between blood flow and disease progression, these flow quantification techniques can have major ramifications for both diagnosis, treatment and follow-up of vascular pathologies, paving the way to true personalized medicine.
Conclusion

Recently, multiple engineering approaches have made significant progress in the quantification of blood flow in peripheral arteries. Although most of the described techniques require further development before they can be used effectively in a clinical setting, they carry great potential to improve the outcomes of vascular procedures.
Influence of Iliac Stenotic Lesions on Blood Flow Patterns Near a Covered Endovascular Reconstruction of the Aortic Bifurcation (CERAB) Stent Configuration

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Abstract

Purpose: To investigate the effect of distal stenotic lesions on flow patterns near a covered endovascular reconstruction of the aortic bifurcation (CERAB) configuration used in the treatment of aortoiliac occlusive disease.

Method: Laser particle image velocimetry measurements were performed using in vitro models of the aortic bifurcation with and without a CERAB configuration in place. A hemodynamically nonsignificant stenosis (ΔP: 9 mm Hg), a hemodynamically significant (ΔP: 26 mm Hg) stenosis, and a total occlusion were simulated in the left iliac arteries. Velocity fields and time-averaged wall shear stress (TAWSS) were calculated.

Results: Hemodynamically significant distal lesions did not influence the inflow patterns or TAWSS (0.5–0.6 Pa) in either model. However, hemodynamically significant distal stenotic lesions caused a 2-fold decrease in peak outflow velocities (control: 106 vs 56 cm/s, CERAB: 96 vs 54 cm/s) and a 3-fold decrease in TAWSS (control: 1.34 vs 0.44 Pa, CERAB: 0.75 vs 0.21 Pa). There was a 2-fold decrease in wall shear stress in the CERAB outflow compared with the control, independent of lesion severity.

Conclusion: In the CERAB technique, adequate distal runoff is identified as an important parameter to ensure patency. This in vitro study showed that distal stenotic lesions influence aortic bifurcation outflow patterns and TAWSS more extensively in the CERAB configuration. Distal stenotic lesions could therefore increase the risk of disease progression and loss of stent patency. In vivo studies are necessary to confirm these observations.

Introduction

Traditionally, the treatment of choice for extensive aortoiliac occlusive disease has been open surgery, while the kissing stents technique has been applied in the last decades for less complex lesions. A recent alternative therapy for extensive disease is the covered endovascular reconstruction of the aortic bifurcation (CERAB) technique [132]. Satisfying early results have been reported, with primary and secondary patency rates of 87% and 95%, respectively, at 2 years’ follow-up in a group with >80% TransAtlantic Inter-Society Consensus (TASC II) D lesions [132]. This patient selection makes it difficult to compare the results to studies of other endovascular techniques that have included fewer type D lesions.
In an in vitro setup, our group recently showed that regional flow patterns around the CERAB configuration are more comparable to physiologic flow than those in bare metal or covered kissing stents [58]. Pathophysiologic wall shear stress (WSS) at the in- and outflow of the configurations are also of interest as WSS may promote the development of atherosclerotic plaque and, in turn, could affect stent patency due to the occurrence of restenosis [49]. However, it is still unclear how these flow patterns and WSS values are influenced by stenotic lesions in the iliac artery distal to the stent configuration. These lesions may cause poor runoff, which has been identified as a risk factor for stent failure [28]–[30], [133]. A previous study evaluating the CERAB technique also suggested that outflow obstructions distal to a CERAB reconstruction could reduce primary patency [132]. Therefore, this study investigated the influence of distal stenotic lesions on blood flow patterns and quantified WSS values near the CERAB configuration.

Figure 1. Covered endovascular reconstruction of the aortic bifurcation (CERAB) using Atrium V12 stents placed in an in vitro infrarenal aorta model filled with blood mimicking fluid (BMF). The white polytetrafluoroethylene covers were replaced by transparent polyurethane to obtain optical access. The black lines indicate the vessel walls, which are otherwise invisible due to refractive index matching between the silicone vessel phantom and the BMF. u and v represent the direction components of the velocity vector. The 2-headed arrow indicates the distance between the anatomic bifurcation and the neobifurcation.
Methods

Model Design and Experimental Setup
Laser particle image velocimetry (PIV) measurements were performed to obtain velocity vector fields of a rigid silicone model of the abdominal aorta with renal arteries, common iliac arteries (CIA), and a CERAB configuration in place. The CERAB configuration (figure 1) was constructed in-house using a platform based on the fully covered Advanta V12 stents (Atrium Maquet Getinge Group, Mijdrecht, the Netherlands) [58]. Two 8-mm Atrium V12 stents (the limbs) were placed within the distal part of a funnel-shaped, tapered V12 stent (16 mm proximally tapering to 12 mm distally). The neobifurcation started ~20 mm proximal to the anatomic bifurcation (figure 1). The white polytetrafluoroethylene covers were replaced by transparent polyurethane to obtain optical access. To simulate stenoses for some of the experiments, 1-cm-long inserts with reduced diameters were placed in the 8-mm outflow tube representing the left CIA. Measurements performed in the CERAB model were compared to a control model without stents.
The flow models and experimental setup were previously described [14], [58], [134] and are shown in Figure 2. Systolic and diastolic blood pressures were set to 130 and 90 mm Hg, respectively. A heart rate of 60 beats per minute and a mean 1.6-L/min suprarenal inflow, divided equally over both renal and both iliac vessels, created physiologic pulsatile flow conditions (figure 3) [135].

Light from a continuous wave 532-nm laser (Cohlibri; LIGHTLINE lasertechnik GmbH, Osnabrück, Germany) was transformed into a laser sheet (40-mm width, <1-mm thickness) using cylindrical lenses. The laser sheet illuminated the central plane of the model. A high-speed camera (Fastcam SA-X2; Photron Inc, Tokyo, Japan) captured the fluorescence from the 1- to 20-µm tracer particles (Dantec Dynamics A/S, Skovlunde, Denmark) during 12 heartbeats at 1000 frames per second (fps) at the inflow and at 2000 fps at the outflow of the CERAB configuration (the outflow contained higher peak velocity values, requiring a higher frame rate). The frame rate was chosen to maximize the correlation peaks of particle displacement with respect to the observed velocities of the region of interest (ROI). A long-pass filter, with a cutoff wavelength of 625 nm, was used to filter out reflecting laser light at 532 nm.

Measurements
Images were captured in 2 ROIs at the inflow (distal aorta) and outflow (left CIA), depicted as positions 1 and 2 in Figure 2. Four measurements were performed in each ROI using modified iliac outflow conditions (a healthy distal artery, a hemodynamically nonsignificant stenosis, a hemodynamically significant stenosis, and a total occlusion) in both the control and CERAB models (Table 1). Total occlusion was simulated by clamping the vessel only for the inflow ROI measurements because during total occlusion, no flow and consequently no shearing force were present in the left CIA.
Pressure gradients across the stenotic lesions were measured with a built-in pressure sensor (40PC015G1A; Honeywell International Inc, Morris Plains, NJ, USA) distal to the stenosis location. Systolic pressure at the bifurcation was used to calculate the pressure difference. The obtained pressure gradients across the stenotic lesions corresponded with physiologic observations [136], [137]. The experiments were run twice using the same setups and conditions; the results of each sample location were averaged.

Figure 2. Schematic representation of the experimental setup, where 1 is the inflow region of interest (ROI) and 2 is the outflow ROI. The dashed lines indicate the contours of the covered endovascular reconstruction of the aortic bifurcation (CERAB) deployed in the vessel phantom. The setup was based on a second-order Windkessel model. The inlet section (~100 cm) ensured fully developed flow entering the model. Peripheral resistance was controlled with needle valves. The compliance distal to the vessel phantom was used to model the peripheral dispensability of the vessels. The circulating blood mimicking fluid was a mixture of water (47.4%), glycerol (36.9%), and sodium iodide (15.7%). The resistance was added to mimic an outflow stenosis.

Pre- and postprocessing was performed using MATLAB (MathWorks Inc, Natick, MA, USA). PIV analysis was performed through pairwise cross-correlation in all captured images using the PIVlab tool (version 1.4) for MATLAB. Velocity vector fields during peak systolic velocity (PSV) and end-systolic velocity (ESV) were obtained. WSS was calculated as $\mu \frac{\delta u}{\delta y}$ where $u$ is the velocity along the vessel wall, $y$ is the height above the vessel wall, $\frac{\delta u}{\delta y}$ is the flow gradient perpendicular to the vessel wall, and $\mu$ is the dynamic viscosity of the blood mimicking fluid (BMF; Figure 1). WSS analysis was performed for the wall segments captured in each ROI through interpolation of the velocity data at multiple nor-mal vectors, perpendicular to the vessel wall using the no-slip boundary condition for viscous fluids. Time-averaged WSS (TAWSS) was plotted against vessel length; TAWSS values are shown for the left aortic wall in the inflow ROI and the lateral iliac wall in the outflow ROI. Similar TAWSS values were found at the opposite vessel walls. WSS analysis was less accurate inside the CERAB configuration (and not relevant in this study) and was therefore not evaluated.
Figure 3. Suprarenal flow pattern used as input for the model. The flow profile that was used to control the gear pumps is in red and the measured flow at the inlet section of the setup is in blue.

<table>
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Table 1. Measurements Obtained for Regions of Interest in Both Models. # = measurement number.

Results

Inflow ROI
Similar undisturbed flow patterns were seen in the inflow ROI of the control model and CERAB model with a healthy distal artery during both PSV and EDV. During ESV, back-flow velocities near the vessel walls were higher compared to the velocities in the center of the vessel lumen in both models (figure 4, bottom).
The flow pattern in the inflow ROI of the control model was not influenced by distal stenosis or occlusion. In the CERAB model, a total occlusion of the distal artery caused an asymmetric flow during ESV but not during PSV (figure 4, right). During ESV, backflow from the unaffected CIA was jetted into the proximal CERAB cuff; concurrent recirculation was observed.

In both models, distal stenosis (both nonsignificant and significant) did not influence TAWSS (figure 5). In the control model, a 20% decrease in TAWSS was observed in a total occlusion of the left CIA. This was not seen in the CERAB configuration (figure 5).

Figure 4. Flow velocity vector fields at the inflow of the covered endovascular reconstruction of the aortic bifurcation (CERAB) model with a healthy distal artery (left) and total occlusion (right) during peak systolic velocity (PSV; upper) and end-diastolic velocity (ESV; lower). Stent mesh indicated in red. The center graph shows axial velocity profiles for both the healthy distal artery and total occlusion situation in the CERAB model during PSV.
Figure 5. Time-averaged wall shear stress (TAWSS) at the left aortic vessel wall in the inflow region of interest (ROI) of the control and covered endovascular reconstruction of the aortic bifurcation (CERAB) model. The dashed line represents the proximal edge of the CERAB cuff.

**Outflow ROI**

In the setup with healthy distal arteries, flow velocity during PSV in the proximal part of the outflow ROI was 40% lower in the control model than in the CERAB model (57 vs 95 cm/s). In the distal part of the ROI, similar peak flow velocities were seen in the control model and the CERAB model (figure 6). In the outflow ROI, a more developed, parabolic axial flow profile was present in the CERAB model compared with control, with lower flow velocities near the vessel wall (figure 7), which caused a smaller velocity gradient with regard to the control.

In both the CERAB and control models, a decrease in velocity during PSV was seen across the vessel lumen with increasing stenosis severity (figure 7). The nonsignificant stenosis caused a small drop in PSV, while a significant stenosis caused 53% and 56% decreases in the 2 experiment runs (control: 106 vs 56 cm/s, CERAB: 96 vs 54 cm/s).

In the control model, an earlier onset of backflow or flow reversal (FR) was seen with increased stenosis severity (FR in Figure 8). The second moment of FR (back to forward flow) remained the same, indicating that distal stenosis caused a longer duration of backflow. This effect was not observed in the CERAB model.

In both the stented and control models, TAWSS in the outflow ROI decreased due to distal stenosis (figure 9). In the models with a significant stenosis, there was a 3-fold decrease in TAWSS compared to the models with healthy distal arteries (average 2–4 cm; control: 0.44 vs 1.34 Pa, CERAB: 0.21 vs 0.75 Pa). In the CERAB model, TAWSS was 2 times lower than in the control model, independent of stenosis severity (average 2–4 cm; healthy: 0.75 vs 1.34 Pa, significant stenosis: 0.21 vs 0.44 Pa).
Figure 6. Velocity vs time plot of outflow region of interest (ROI) in the control and covered endovascular reconstruction of the aortic bifurcation (CERAB) model. Locations of velocities are indicated by the gray and purple dots in the top right panels.

Figure 7. Flow velocity vector fields of the outflow region of interest in the control (upper) and covered endovascular reconstruction of the aortic bifurcation (CERAB; lower) models during peak systolic velocity (PSV). The graphs show axial velocity profiles for the 3 stages of distal outflow stenosis in both the control (upper) and CERAB (lower) model during PSV.
Figure 8. Velocity vs time plots of outflow region of interest in the control and covered endovascular reconstruction of the aortic bifurcation (CERAB) model. Locations of velocities are indicated by the red dots in the top right panels. FR, moment of flow reversal.

Figure 9. Time-averaged wall shear stress (TAWSS) in the outflow region of interest (left iliac artery, lateral wall) of the control model and covered endovascular reconstruction of the aortic bifurcation (CERAB) model. Length 0 is just distal of the bifurcation. The dashed line represents the distal edge of the left CERAB limb.
Discussion

This study has shown that the inflow section of the CERAB and control models are not influenced by outflow stenosis, but an occlusion causes jetting from the nonoccluded limb into the cuff of the CERAB, and a 20% decrease in TAWSS occurs in the control model. In the inflow ROI, the highest velocities during ESV were found near the vessel wall. This was caused by backward flow from the distal aorta toward the renal arteries during ESV. Holenstein and colleagues [138] attributed the occurrence of backflow to differences in resistance between the renal system and peripheral system of the legs. Velocities near the vessel wall also returned to a forward direction earlier than in the center of the vessel, indicating the presence of a Womersley velocity profile [139]. These phenomena are also known to occur in vivo, which confirms the similarity between the flow conditions in human physiology and the models used in the study.

A 40% decrease in flow velocity measurements during PSV was observed in the control model at the anatomic bifurcation vs the same location in the CERAB model. This can be explained by the fact that the CERAB limbs keep the flow lumen at 8 mm starting at the neobifurcation, whereas the native bifurcation widens before it narrows into the CIA (branch to trunk ratio >1). This influences the observed flow profiles and TAWSS values in the distal iliac vessels.

The observed flow profile in the control model was more blunt or plug-shaped, while in the CERAB the profile was more parabolic, causing a smaller velocity gradient and resulting in lower TAWSS values. This can be attributed to the fact that the outflow profile of the CERAB can develop (ie, become more parabolic) over a longer distance (~20 mm) compared with the control. Therefore, prior to the introduction of a distal stenosis, the TAWSS near the CERAB outflow was already 2 times lower compared with the control model.

Introducing stenosis in the distal outflow section of both control and CERAB models caused a 2-fold decrease in flow velocity and a 3-fold drop in TAWSS values. Several studies have previously shown that areas with low TAWSS correspond to regions that may exhibit atherosclerotic lesions, leading to the belief that low TAWSS causes an increased risk of plaque formation [52], [53], [140]. In the CERAB reconstruction, this means that low TAWSS due to a distal stenosis could increase the risk of new or progressive plaques and therefore may accelerate disease progression, with potential detrimental consequences for stent-graft patency.

In the outflow tract of the CERAB configuration, a 2-fold decrease in WSS was seen compared to the control model, independent of the presence or severity of a distal stenotic lesion. This indicates that the risk of accelerated disease progression due to distal stenosis might well be greater in patients treated with a CERAB compared with untreated patients. Progression of atherosclerosis can lead to failure of the CERAB configuration, which may lead to acute limb ischemia. Besides the possible mid- to long-term effects,
more acute modes of failure could be attributed to the fact that low WSS also increases the risk of thrombosis [55]. Our group encountered early thrombosis in 2 patients 3 weeks after CERAB reconstructions [141].

A previous study by Grimme et al [1] showed that outflow impairment near the CERAB configuration can cause occlusion of the stents and should therefore be monitored intensively and treated early [132]. The current study provides in vitro local hemodynamic data that suggest stenotic lesions more distal to the CERAB configuration could have the same effect. Treatment of this type of lesion, when significant, should therefore be considered in patients with a CERAB configuration, regardless of the patient’s symptom status. In the case of nonsignificant stenosis, an increase in follow-up intensity should be considered. Placement of the stent configuration should likely be combined with femoral endarterectomy in patients with a preexisting stenotic lesion around the femoral bifurcation [142].

The lower WSS values in the outflow tract of the CERAB configuration seem to be related to the distal leg rather than the proximal cuff. This finding could also apply to other stent configurations that create a neobifurcation, such as kissing stents or grafts used for open surgery, as the extension of the iliac lumen gives rise to a more developed flow profile, with lower TAWSS values. A previous in vitro study by Walker et al [143] also showed lower WSS downstream of a stent wire, lending credibility to this assumption. This would mean that treatment of stenosis distal to a stent should be considered in all patients, not only in patients with a CERAB configuration.

Besides WSS, the oscillatory nature of WSS, reported as the oscillatory shear index (OSI), also correlates with the progression and development of atherosclerosis [144]. Higher OSI values indicate a balanced variation between the positive and negative directions of WSS. The metric was not included in these results because the observed pattern depicted the inverse of the WSS curves. The PSV decreased (positive WSS) with increasing stenosis severity, balancing with the backflow (negative WSS) component and causing an increase in OSI.

**Limitations**

Several limitations inherent to in vitro studies influenced the results of this study, such as the compliance of the vessels, interaction between the stent and vascular endothelium, and movement of the vessels, which were not modeled in the in vitro setup. Stent covers made of transparent poly-urethane were used to simulate the geometry of the CERAB for obtaining optical access. There was no attempt to mimic the material properties of expanded polytetrafluoroethylene. Furthermore, direct measurement of WSS using PIV techniques is not possible. Therefore, WSS values were always based on the velocity profile normal to the wall, which can be obtained only at a finite distance from the wall. This necessitates an interpolation of the flow profile to the wall, possibly introducing a measurement error. A high spatial and temporal resolution in combination with accurate wall detection is therefore needed to calculate the WSS with reasonable accuracy.
Lower WSS distal to the stents, such as observed in this in vitro study, may not be present in vivo, for example, due to the reorganization of endothelial cells around the stent edges and the compliance of arteries. Thus, in vivo studies to quantify blood flow patterns around stents are required. It should be noted, however, that in vivo flow quantification, with high spatial and temporal resolution, is not a simple matter. Aside from resolution constraints, magnetic resonance imaging–based techniques for in vivo flow quantification in stents create considerable material-dependent artifacts that may inhibit quantitative analysis near the stent configuration [105]. Contrast-enhanced, high-frame-rate, ultra-sound-based techniques (echoPIV) could prove to be a convenient modality to quantify the in- and outflow of stent configurations. Until now, echoPIV techniques have been used only in superficial vessels and cardiac applications in a research setting [145]. Furthermore, clinical follow-up studies should be performed to investigate the effect of WSS on stent patency and subsequent clinical outcome.

Another relevant difference between the in vitro model and human physiology is the iliac outflow tract of the measurement setup. Both iliac vessels were connected to a single compliance and resistance. Therefore, during measurements, a decreased flow in the stenotic vessel automatically led to an increased flow in the contralateral vessel. In human physiology, the demand of oxygen is regulated for each organ individually by adjusting vascular resistance. Thus, in principle, a different redistribution of flow could take place. However, the redistribution of flow through the entire body due to a stenosis in a single vessel is complex and was therefore not simulated in this study. Furthermore, a left-right comparison was not attempted. However, a future study accurately modeling the flow distribution would be of interest, and the addition of a contralateral stenosis would add further strength to the analysis.

**Conclusion**

This in vitro study shows that stenotic lesions distal to the aortic bifurcation cause a decrease in peak flow velocities and a corresponding decrease in TAWSS in the outflow tract of both the control and CERAB models. In the outflow tract of the CERAB configuration, TAWSS was lower than in the control independent of the severity of the distal stenotic lesion, indicating that local accelerated disease progression could be greater in patients treated with the CERAB technique. Therefore, treatment of stenotic lesions distal to a CERAB configuration could be indicated regardless of the presence or absence of symptoms. In vivo assessment of local flow patterns in combination with patient follow-up is required to verify these findings.
Chapter 4
High Frame Rate Contrast-Enhanced Ultrasound for Velocimetry in the Human Abdominal Aorta

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Abstract

**Introduction**: Treatment of abdominal aortic (AA) aneurysms and stenotic lesions may be improved by analyzing their associated blood flow patterns. Angle-independent blood flow patterns in the AA can be obtained by combining echo-particle image velocimetry (echoPIV) with high frame rate contrast-enhanced ultrasonography. However, echoPIV performance is affected by ultrasound contrast agent (UCA) concentration, microbubble stability and tissue clutter.

**Method**: In this study we assessed the influence of acoustic pressure and UCA concentration on image quality for echoPIV analysis. We also compared amplitude modulation (AM) and singular value decomposition (SVD) as tissue suppression strategies for echoPIV. Fourteen healthy volunteers were imaged in the region of the distal AA. We tested four different UCA bolus volumes (0.25, 0.5, 0.75 and 1.5 ml) and four different acoustic output pressures (mechanical indices: 0.01, 0.03, 0.06 and 0.09). As image quality metrics, we measured the contrast-to-background ratio, bubble disruption ratio and maximum normalized cross-correlation value during echoPIV.

**Results**: At mechanical indices $\geq 0.06$, we detected severe bubble destruction, suggesting that very low acoustic pressures should be used for echoPIV. SVD was able to suppress tissue clutter better than AM. The maximum tracking correlation was affected by both UCA concentration and flow rate, where at high flow rates, lower UCA concentrations resulted in slightly higher correlation values, but more signal drop-outs during late diastole.

**Conclusion**: High frame rate echoPIV was successfully performed in the AA of healthy volunteers and shows promise for future studies in patients.

Introduction

Study of abdominal aortic (AA) flow patterns may assist the disease-progression prediction process in patients with AA stenotic lesions and aneurysms. Several studies on stenotic lesions suggest that local flow patterns and their associated flow parameters, such as wall shear stress, have an influence on lesion development and progression [48], [52], [54]. In AA aneurysms, changes in flow patterns modulate inflammatory mechanisms in the vascular endothelium, causing aneurysm growth [146]. For AA aneurysms and stenotic lesions around the aortic bifurcation, in vitro data have shown that different treatment options generate different flow perturbations [58], [147], which can partly explain the different outcomes of these treatments. Post-treatment analysis of AA blood-flow patterns may make the follow-up schemes after endovascular treatment more patient-specific by predicting potential failure.
Investigation of blood flow patterns in vivo requires full field, angle independent velocity measurements. Currently, the most widely used method of assessing AA blood flow is Doppler ultrasound. However, conventional Doppler is angle dependent, which complicates imaging blood flow in regions of bifurcation, where blood flows in different directions, and where it can also flow approximately perpendicular to the ultrasound beam (70° to 110°) [63], [148].

Several ultrasonic techniques have been developed to overcome the angle dependency limitations of standard Doppler. Vector Doppler Imaging (VDI) splits the transmit aperture, obtaining multiple Doppler measurements at known angles to each other, from which both velocity magnitude and direction can be deduced [71], [149]. However, for imaging of deep structures, the angles between beams, and hence velocity estimates, can become unreliable due to the limited aperture [150]. Transverse Oscillation is a technique that also utilizes a split aperture, although usually only synthetically in receive [73]. Although originally limited to linear arrays, this method has recently been expanded to work with curved arrays, being demonstrated in the portal vein of a healthy volunteer [151]. However, the velocities expected in the AA are much higher than those in the portal vein. Blood Speckle Tracking can obtain angle-independent velocity measurements by tracking the speckle motion of moving red blood cells between frames [152]. It, however, requires sufficient temporal resolution for tracking the range of flows expected in the AA. High frame rate (HFR) imaging, using unfocussed transmissions, allows for the temporal resolution required for tracking high blood flow velocities, but is complicated by strong clutter in the blood-pool from surrounding tissue and reduced penetration depth compared to focused transmissions [153].

Echo-particle imaging velocimetry (echoPIV) using ultrasound contrast agent (UCA) can be beneficial for the penetration depths required in AA flow imaging in patients (6-10 cm), since backscattered signal is greatly improved over native blood cells. We have shown previously that HFR echoPIV can accurately measure the high velocity flows which are expected in the AA, in vitro [154]. Translation to in vivo, however, requires further optimization of critical UCA related parameters, such as mechanical index (MI), UCA concentration and the applied tissue suppression strategy.

UCA specific acquisition sequences suppress tissue signal by exploiting the non-linear behavior of UCA, e.g. amplitude modulation (AM) or pulse inversion. However, these sequences incur a cost in frame rate, as multiple transmissions are required to reconstruct a single image. Alternatively, singular value decomposition (SVD) based tissue suppression has been shown to perform equivalently or better than UCA specific acquisition sequences, although only for microvascular flow environments [155]. It is not yet known whether SVD also performs well in large vessels like the abdominal aorta.
The use of UCA also mandates careful tuning of the acoustic pressures used for imaging. Too-low pressures may generate insufficient signal from the bubbles; while overly-high pressures can result in bubble destruction. In both cases, velocity estimation will be compromised. The relationship between acoustic pressure and bubble destruction during HFR imaging has been reported only for in vitro studies [37], [156], [157]. It is well known that bubble stability is affected by physiological conditions. In this study we assess bubble destruction in vivo.

Another variable requiring optimization is UCA concentration. Higher concentrations are associated with higher signal power, but may reduce echoPIV accuracy if too high [158], [159]. Conversely, low concentrations may leave void regions, occupied only by noise. The effect of UCA concentration has not yet been studied for HFR echoPIV.

In this study, we investigate the effect that tissue suppression strategy (AM versus SVD), acoustic pressure and UCA concentration have on image quality metrics for echoPIV, in human volunteers.

Methods

A. Study Design

After approval as a pilot study, by the medical ethic committee of the Erasmus Medical Center (NL58025.078.16), 15 healthy volunteers (age 18-35 years, BMI <25) were imaged in the region of the distal aorta with the aortic bifurcation and proximal iliac vessels in a coronal view. Four bolus injections of UCA (0.25, 0.5, 0.75 and 1.5 ml, SonoVue, Bracco S.p.A., Milano, Italy) were administered before acquiring 2.5 s of HFR ultrasound data with a research ultrasound system (Vantage 256, Verasonics Inc., Kirkland WA, USA).

An additional clinical ultrasound system (Epiq 7, Philips Healthcare, Andover, MA, USA) was used to simultaneously record contrast mode image sequences in the left superficial femoral artery (downstream of the AA). HFR recordings in the AA were initiated on the research ultrasound system once the bolus was detected in the femoral artery.

After imaging the first volunteer, some minor adjustments/improvements were made to the acquisition scheme, making the data of this volunteer incomparable with the others. Measurements were performed on the remaining 14 volunteers during four measurement sessions (afternoons) in groups of 3-4. The first three volunteers were imaged at a transmit voltage of 30V. Due to clearly visible bubble destruction on the clinical system, the transmit voltage on the Verasonics ultrasound system was decreased for subsequent volunteers, after each measurement session. Thus, three volunteers were imaged using a transmit voltage of 30V, three at 20V, four at 10V and four at 5V. The transmit voltages of 30V, 20V, 10V and 5V correspond to MIs of 0.09, 0.06, 0.03 and 0.013, respectively (at a depth of 30-50 mm taking into account a tissue attenuation of -0.3 dB per cm).
Additionally, the volunteers underwent MRI phase contrast imaging and the detected flow was compared to the echoPIV results. This part of the study is not further described here, but reported elsewhere [91].

**B. Ultrasound Acquisition and Image Reconstruction**

RF data were acquired with a curvilinear probe (3 MHz, C5-2, ATL, Bothell WA, USA) connected to the research ultrasound system. The AM sequence consisted of diverging waves (transmit delays all zero, single cycle pulse) transmitted with different apodization schemes (even, full, and odd elements active [160], [161]) at a pulse repetition frequency (PRF) of 3000 Hz. The sum of odd and even apodization transmissions was coherently subtracted from the full transmit to produce AM images at 1000 fps. From the full transmit acquisitions a standard B-mode sequence of 1000 fps was also generated, producing synchronized datasets for comparison. Images were beamformed into the polar domain where further analysis was performed.

**C. Singular Value Decomposition (SVD)**

SVD based clutter suppression assumes that the tissue, blood and noise components of an image sequence can be separated based on their respective spatiotemporal coherence energy [39]. Tissue signal is typically higher intensity and more spatiotemporally coherent than flowing blood (and bubble) signal. Thus, when an image sequence containing blood flow and surrounding tissue is decomposed using SVD, the tissue signal accumulates more coherence energy than the flowing blood. This causes tissue to collect in the low-rank modes of the system while blood and bubbles are distributed more centrally (Figure 1.). Noise, being relatively incoherent and low intensity, typically resides in the high-order modes. Truncating low and/or high order modes allows for selective removal of tissue and/or noise from the image sequence.

In this study, a low-rank threshold selection algorithm was used to automatically detect the transition between tissue and flowing UCA. Low-rank selection was based on the ratio of successive singular values: $\sigma_n/\sigma_{n-1} > 0.99$ (see Figure 1). This criterion selects the first mode $n$ which decreases less than 1% in energy from its predecessor [162]. A high-rank cutoff was not used in this study.

The number of frames used when performing SVD (ensemble length) is known to affect the separability of slow moving bubbles and tissue [155]. Thus, to assess the effect of SVD ensemble length on contrast-to-background ratio (CBR) four different SVD ensemble lengths were tested: 32, 64, 128 and 1250 frames (all frames). CBR was assessed during periods of slow flow (velocity magnitude $< 0.1$ m/s) and fast flow (velocity magnitude $> 0.4$ m/s) separately. Comparison was performed on data with MI = 0.01 only to reduce the influence of bubble disruption on the comparison.
SVD was performed on beamformed IQ data. For ensemble lengths of 32, 64 and 128, individual SVD outputs needed to be combined into a continuous set of frames. Thus, ensembles were overlapped by 87.5%, where overlapping frames from different SVD ensembles were averaged to create the final SVD outputs. This was not required with the 1250 ensemble as only one SVD output was created.

Figure 1. Illustration of the low-rank threshold selection algorithm used in this study. Tissue cutoff is found by searching for the point in the curve where the slope begins ‘flattening out’.

D. Tissue Suppression Strategies
AM was compared to SVD (ensemble length = 1250 frames) as a method for suppressing tissue signal without deteriorating the UCA signal. SVD images were computed from the B-mode sequences. Additionally, a 2nd order Chebyshev high-pass filter with a -6dB cutoff at 15 Hz was applied to the AM data, acting as a low-cutoff frequency Doppler wall-filter (AM+Cheby). SVD was also applied to the AM processed data (ensemble length = 1250 frames) as an additional group for comparison (AM+SVD), to investigate the usefulness of a combination of the two techniques.
**E. Contrast-to-Background Ratio (CBR)**

Tissue suppression efficacy was assessed using contrast-to-background ratio (CBR) [163], defined as $\text{CBR} = 10 \log_{10} \left( \frac{\overline{\text{RMS}}_{B_{1-10}}}{\overline{\text{RMS}}_{A}} \right)^2$, where $\overline{\text{RMS}}$ is the time-averaged root-mean-square signal strength in UCA (figure 3:A). Comparison between AM and SVD was performed during periods of slow flow (mean velocity < 0.1 m/s), which is the worst-case scenario for SVD, where bubble coherence between frames is similar to that of slowly moving tissue, increasing the likelihood that bubble signal will be removed along with the tissue signal.

**F. Disruption Ratio**

UCA disruption ratio (DR), a measure of acoustically driven bubble destruction, was calculated $\text{DR} = 1 - \frac{\overline{\text{RMS}}_{B_{10}}}{\overline{\text{RMS}}_{B_{1}}}$, where $\overline{\text{RMS}}$ is the time-averaged RMS signal in the proximal (figure 3:B1) and distal (figure 3:B10) regions inside the AA. DR values range from 0 to 1, implying no any and full bubble destruction, respectively [20]. DR was calculated on the SVD processed datasets during systole only (mean velocity > 0.4 m/s) to ensure that fresh bubbles were being supplied to the region of interest.

![Figure 2. Regions used for calculating DR and CBR. Red lines indicate outlines of AA and bifurcation to iliac arteries. Purple dotted region A was used for tissue signal strength. Regions B1 to B10 were used for UCA signal strength. B1 and B10 were used for DR. Images displayed at 50dB dynamic range.](image-url)
G. Bubble Concentration / Velocity Tracking
This section describes how the velocity and correlation values were calculated for comparison between different bolus concentrations. Velocity in the center of the vessel was estimated using normalized cross-correlation (along slow-time, frequency domain implementation) in ten regions (Figure 3: B1-10) running along the length of the vessel. Each region was 4.7° by 6 mm in size, resulting in regions sized approximately 6 mm by 6mm, once scan converted. This size was chosen to meet the widely accepted ¼ interrogation window rule for PIV [41]. Normalized cross-correlation was performed on the polar beamformed data after envelope detection. The maximum correlation value was used as a measure of tracking performance for different UCA concentrations. Velocity vectors were determined by finding the location of maximum cross-correlation per region (Figure 3. B1-10). Subpixel displacement was estimated using the centroid approach [41]. Velocity vectors were scan-converted and then smoothed using a temporal moving median filter (15 ensemble length). Bubble concentrations during diastolic (mean velocity < 0.1 m/s) and systolic (mean velocity > 0.4 m/s) phases were assessed separately, where maximum normalized cross-correlation and CBR were used for comparison.

H. echoPIV Measurement
A full echoPIV measurement is demonstrated on a volunteer imaged at 0.01 MI with a bolus volume of 1.5 ml, after applying a 1250 ensemble SVD filter. Four cross-correlation iterations were performed with window deformation, using interrogation areas of 9.5° x 6.1 mm and an overlap of 75% [41]. Correlation compounding was performed on three subsequent frame pairs before subpixel displacement estimation using a centroid approximation [41]. Vector fields were processed for display - at peak systole, backflow and diastole - using the dynamic visualization procedure described in [164]. Vessel boundaries were manually segmented.

I. Statistics and Reporting
Significance of differences was statistically tested using a two-tailed Student’s t-test, where a p-value < 0.05 implied significance. Results are reported as mean ± standard deviation.
For box plots: circles denote individual data points; whiskers extend to max and min values of non-outliers; boxes start and stop at first and third quartiles; solid lines denote median; and dashed lines denote mean (if present).
Figure 3. CBR values obtained with different SVD ensemble lengths during periods of slow and fast flow. Longer ensembles achieve higher CBR during slow flow. During fast flow, the opposite is true. Only 0.01 MI data was used for this comparison. n = 16 (volunteers x bolus volumes). * p < 0.05

Results

Ultrasound contrast agent (UCA) was detected in all volunteers using HFR ultrasonography with no adverse events. UCA signal could be detected using all of the tissue suppression strategies tested.

A. SVD Ensemble Length
Increasing SVD ensemble lengths resulted in increasing CBR during periods of slow flow (figure 3). However, during periods of fast flow shorter ensembles resulted in higher CBR.
Figure 4. Effects of MI on CBR of AM images and DR. a) Increasing MI results in lower CBR for AM processing. At MI ≤ 0.03 larger bolus volumes result in more CBR. b) Tissue-signal intensity after AM processing increases quadratically with MI (dashed line indicates quadratic fit). c) Increasing MI results in more bubble destruction, where horizontal bars denote non-significant differences between groups. Numbers represent sample size (volunteers x bolus volumes).

B. Mechanical Index (MI)
For AM processed data, increasing MI resulted in reduced CBR (figure 4.a). Larger bolus volumes resulted in higher CBR but only for the lower MIs (0.01 and 0.02 - Figure 4.a). The tissue signal after AM processing increased quadratically with increasing MI (Figure 4.b). Higher MIs (0.06 and 0.09) caused considerably more microbubble destruction than lower MIs (figure 4.c). Contrast-ultrasound recordings in the femoral artery, downstream from the HFR imaged AA, showed dips in intensity during HFR insonification for the higher MIs but not for the lower MIs (figure 5).
Figure 5. Contrast mode images recorded downstream from the abdominal aorta, in the left superficial femoral artery with a clinical ultrasound system. Images are recorded a few seconds before (a, c) and during (b, d) the high frame rate (HFR) acquisitions in the abdominal aorta, for MIs of 0.01 (a, b) and 0.06 (c, d). For 0.06 MI, note the dramatic reduction in contrast intensity before (c) and during (d) the HFR acquisition. This is not the case of 0.01 MI, where contrast intensities before (a) and during (b) HFR acquisition are very similar.

C. Tissue Suppression

SVD consistently provided superior CBR values to AM and filtered derivatives of it, for all the MIs tested (figure 6). No significant differences were noted between AM+Cheby or AM+SVD, although both resulted in higher CBR than AM alone, even at 0.01 MI, where AM performed at its best. Frames of each filter group at different MIs are shown during slow flow only (|v| < 0.1 m/s) in Figure 7. The average depth to the centerline of the aorta observed in these volunteers was 32±5 mm.
Figure 6. CBR values for increasing MI and different contrast enhancement schemes (clutter filters). SVD is consistently superior to AM, AM+Cheby and AM+SVD. Note that while AM CBR reduces with increasing MI, AM+Cheby and AM+SVD do not. CBR calculated during periods of slow flow only (velocity < 0.1 m/s). Numbers represent sample sizes (volunteers x bolus volumes).

D. Bubble Concentration
Correlation between frames during fast flow (0.3 ± 0.05) was weaker than during slow flow, independent of UCA concentration (0.7 ± 0.1, Figure 8.a). The 0.25 ml bolus had a lower correlation during slow flow than the 1.5 ml bolus (0.65 ± 0.14 vs. 0.79 ± 0.05, p=0.03) but a higher correlation during fast flow (0.35 ±0.04 vs. 0.30 ± 0.02, p=0.007). Larger bolus volumes increased CBR for both diastolic and systolic flow rates (figure 8b), where systolic CBR was higher than diastolic on average (23±5 dB vs 18 ± 5 dB, respectively, p < 0.001). For the 0.25 ml bolus volumes, signal ‘drop-outs’ were observed towards the end of diastole, where bubble signal was lost in small regions. This was less prominent in higher concentrations.

E. echoPIV Measurement
Taking into account the optimization described in previous sections, echoPIV vector-fields were derived from a volunteer with an MI of 0.01 and a UCA bolus of 1.5ml. The results are shown in Figure 9.
Figure 7. AM, AM+Cheby, AM+SVD and SVD processed frames during slow diastolic flow (<10 cm/s) for the MIs studied (different volunteers). Bolus volume was 1.5 ml. Red lines indicate vessel boundaries. Higher MI results in higher AM tissue signal power and increased bubble destruction (left column to right column). SVD processing produces higher CBR than AM and its filtered derivatives. Images displayed at 50dB dynamic range and normalized individually.
Figure 8. Effect of increasing UCA concentration (bolus volume) on a) maximum normalized cross-correlation values and b) CBR. During fast flow, low concentrations result in slightly stronger correlation between frames than high concentrations. However, during periods of slow flow the opposite was true. Numbers represent sample sizes (number of volunteers).

Discussion

High frame rate contrast enhanced ultrasonography was successfully performed in the abdominal aorta of healthy volunteers. Velocity field information could be determined using echoPIV (with the optimization described in this study) which was very similar to 4D phase-contrast magnetic resonance imaging [91].

A. SVD Ensemble Length

Longer ensemble lengths resulted in increased sensitivity to slow moving bubbles. This was expected as using more frames allows for more time for slow-moving bubbles to develop differences in spatial-temporal coherence from the slow-moving tissue. We also observed that shorter ensemble lengths resulted in higher CBR values for fast flow; this may be due to shorter ensembles being able to remove the pulsatile motion of the vessel wall better than long ensembles. However, for AA applications longer ensemble lengths are preferable as their CBR is best during slow flow and sufficient during fast flow.
Figure 9. echoPIV derived velocity fields during three phases of the cardiac cycle: a) peak systole, b) backflow, and c) diastole. Results obtained with 0.01 ml, 1250 SVD ensembles and 1.5 ml UCA bolus.
B. Mechanical Index (MI)

1) Contrast-to-Background Ratio (CBR)
Lower MIs resulted in higher CBR values for AM processing (Figure 4.a). The reason is two-fold: 1) higher MI results in more bubble destruction (figure 4.b); and 2) higher MI accompanied higher tissue signal (figure 4.c), even after removal of the linear signal component. The reason for the increased tissue intensity is likely non-linear propagation of the pressure wave through tissue, which increases quadratically with the ultrasonic pressure applied [165]. We also observed apparent bubble signal below the AA (figure 7), possibly caused by non-linear propagation through the UCA filled AA, as described in [166], [167].

2) Disruption Ratio (DR)
We observed some differences in bubble destruction to those reported by in vitro studies. Couture et al. [37] reported more than 75% DR at peak-negative pressures of 0.2 MPa (~MI of 0.01 at 7.5 MHz), whereas we observed ~ 20% DR at a MI of 0.01. However, exposure time to ultrasound (~80 ms here versus 25 s used in their study) and acoustic frequencies used (3 MHz versus 7.5 MHz) were drastically different between our two studies. To the contrary, Toulemonde et al. [157] observed negligible bubble destruction at a MI of 0.1. However, their MI values were measured close to the probe, whereas here (and in [37]) MI was measured at the depth of interest (30 mm here and 20mm in [37]). Finally, in vitro studies do not typically account for physiological temperatures [168]–[170] and pressures [171] gas exchange between blood and UCA [172], [173] or filtration by the lungs. We found that a maximum MI of 0.03 could be used without severe bubble destruction. However, it is important to note that DR was established during periods of fast flow; during slow flow, the contrast bubbles will be exposed several times longer to ultrasound resulting in more severe bubble destruction in a given region. Therefore, the lowest MI is preferred. In further research, even lower MI values could be tested.

C. Amplitude Modulation vs. Singular Value Decomposition
SVD achieved higher CBR values than AM (figure 6 and Figure 7). Even when combined with a very ‘mild’ wall filter (AM+Cheby), AM performed worse than SVD. We also tested how applying SVD to AM processed images would compare to SVD on a B-mode image. From Figure 7c, it appears that AM+SVD provides higher signal intensities. However, Figure 6 shows that SVD alone provides higher CBR values than AM+SVD. AM processing reduces the signal level and introduces additional noise during the coherent subtraction process of the AM sequence, which both deteriorate CBR. Although SVD performed well on this data, with small amounts of non-rigid tissue motion, it may not perform so well where tissue motion is relatively large, e.g. the motion of the heart valves and wall in echocardiography.
CBR is not the only factor worth considering in the comparison between AM and SVD. AM needs at least two transmissions to produce an image; we implemented a commonly used three-transmission sequence which overcomes a limitation in the research ultrasound system to quickly switch between different transmit voltages. SVD can be applied to single transmission sequences, as performed in this study. Thus higher frame rates can be achieved when using SVD alone, or angular compounding can be used to reduce side-lobe levels, increasing both resolution and contrast [174]. However, it should be noted that coherent compounding of angular transmissions in the presence of fast moving scatterers is not straightforward, as decorrelation of the scatterers between different angles causes strong imaging artefacts [175]. Alternatively, for echoPIV applications, the compounding of individual angles can be performed in the correlation domain [176], [177].

D. UCA concentration
The mean correlation values obtained during fast flow were much lower than during periods of slow flow, independent of UCA concentration. This was expected as more bubbles will exit (and enter) the interrogation region as the flow rate increases. Additional factors linked to flow speed, such as large flow gradients or out-of-plane flow can also reduce the correlation value obtained. There are methods to account for these effects: including the use of different size interrogation windows between frames; or the use of iterative block-matching schemes with window offset and/or deformation [41] (as was used to obtain the results in Figure 9).

We found that high UCA concentrations facilitated higher correlation during low flow rates and vice versa. The reason for poor performance of low bubble concentrations during slow flow was likely the lower CBR during slow flow (figure 8b). The CBR decrease during slow flow was likely due to more bubble destruction, caused by the increased ultrasound exposure time. Indeed, we observed distinct regions with signal loss, particularly during late diastole, which were more prominent in the 0.25 ml bolus data than in the 1.5 ml bolus data. Thus, for low concentrations, these signal drop-outs during slow flow may outweigh the small correlation improvements during fast flow, as the drop-outs result in significant tracking error.

The small correlation improvement gained by low UCA concentrations during fast flow is in agreement with in vitro studies using conventional line-scanning ultrasound for echoPIV [158], [159]. Likely caused by less ‘particle-pairs’ being present in an interrogation window which reduces correlation uncertainty in the presence of strong flow gradients.
E. Limitations

This study did not test other non-linear contrast specific tissue suppression strategies, such as pulse inversion or power modulated pulse inversion (PMPI), which may have performed better with a different transducer. However, Desailly et al. [155] reported similar results when comparing SVD with PMPI in a microvascular environment. The volunteers in this study had lower BMI than anticipated in the patients of interest, with relatively superficial aortas (32±5 mm) compared with the depths that can be expected in patients (up to 100 mm, sometimes deeper). However, this preliminary study aimed to prove that HFR echoPIV was possible in the region of the AA bifurcation, and to gain insight into optimal UCA parameters for future patient studies. The acoustic pressures required to obtain sufficient signal were also very low, thus the transmit power can be increased to obtain similar MIs in deeper regions. How echoPIV is affected by the increased attenuation and reduced image quality in patients will be assessed in future studies.

We tested lower MIs only after discovering that the planned MI of 0.09 (derived from previous in-vitro studies) was causing severe bubble destruction in vivo. This forced a parameter adjustment for the following batches of volunteers, but allowed us to assess the influence of MI, which was beneficial for the final outcome. For future HFR CEUS studies, in vivo, one should be prepared to use very low MI, maybe even lower than the values used here.

The use of the normalized cross-correlation value as a surrogate for tracking performance is also a limitation of this study, although this is not uncommon [158]. This was required as reliable ground truth measurements were not feasible in vivo.

Finally, the PRF used in this study was not as high as physically possible, but was limited to keep spatial-peak temporal-average intensity (ISPTA) under the recommended value for abdominal imaging [178]. We performed our ISPTA safety measurements to allow for the maximum MI value tested, resulting in an ISPTA value close to the 94 mW/cm² recommended for abdominal imaging. The use of the lower output pressures (MI ≤ 0.01) in this study would allow for a higher PRF in future, possibly up to the physical maximum of ~8000 Hz at a depth of ~10 cm.
Conclusion

We have shown that SVD can provide higher CBR than AM in the abdominal aorta, without requiring multiple transmissions per image. We found that lower MIs should be used in vivo to prevent bubble destruction, as compared to in vitro studies. Finally, we observed that higher UCA concentrations were associated with higher correlation during slow flow conditions and less signal drop-outs, but lower concentrations were associated with slightly higher correlation under fast flow conditions.
High-Frame-Rate, Contrast-enhanced Ultrasound Particle Image Velocimetry in the Abdominal Aorta: first human results

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Hendrik J Vos
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Abstract

**Purpose:** To study the feasibility of high-frame-rate (HFR) contrast material–enhanced (CE) ultrasound particle image velocimetry (PIV), or echoPIV, in the abdominal aorta.

**Method:** Fifteen healthy participants (six men; median age, 23 years [age range, 18–34 years]; median body mass index, 20.3 kg/m² [range, 17.3–24.9 kg/m²]) underwent HFR-CEUS. US microbubbles were injected at incremental doses (0.25, 0.5, 0.75, and 1.5 mL), with each dose followed by US measurement to determine the optimal dosage. Different US mechanical index values were evaluated (0.09, 0.06, 0.03, and 0.01) in a diverging wave acquisition scheme. PIV analysis was performed via pairwise cross-correlation of all captured images. Participants also underwent phase-contrast MRI. The echoPIV and phase-contrast MRI velocity profiles were compared via the calculation of similarity index and relative difference in peak velocity.

**Results:** Visualization of the aortic bifurcation with HFR-CEUS was successful in all participants. Optimal echoPIV results were achieved with the lowest contrast agent dose of 0.25 mL in combination with the lowest mechanical indexes (0.01 or 0.03). Substantial bubble destruction occurred at higher mechanical indexes (≥0.06). Flow patterns were qualitatively similar in the echoPIV and MR images. The echoPIV and MRI velocity profiles showed good agreement (similarity index, 0.98 and 0.99; difference in peak velocity, 8.5% and 17.0% in temporal and spatial profiles, respectively).

**Conclusion:** Quantification of blood flow in the human abdominal aorta with US particle image velocimetry (echoPIV) is feasible. Use of echoPIV has potential in the clinical evaluation of aortic disease.

Introduction

Imaging of endovascular flow patterns in the abdominal aorta is challenging but clinically relevant because of the relationship between local hemodynamics and the development of vascular diseases [56], [57], [179]. Conventional Doppler US enables a one-dimensional blood flow velocity estimate in the axial direction. However, because the aortoiliac bifurcation is perpendicular to the transducer, it is difficult to obtain reliable flow quantification with Doppler imaging.

In the carotid artery and the heart, US particle image velocimetry (hereafter, echoPIV) has been used to obtain two-dimensional velocity vector fields of blood flow in the axial and lateral directions [87], [89]. With this technique, US images are acquired and used for PIV analysis. Recent developments in the use of high-frame-rate (HFR) contrast material–
enhanced (CE) US have improved the possibilities of quantifying blood flow with echoPIV. However, flow velocities of approximately 1 m/sec, which can be found in the human abdominal aorta, have not been successfully quantified until recently [88]. In the abdominal aorta, US is complicated by loss of signal due to bowel gas or imaging depth, which could be compensated by using US contrast agents. However, little is known about the amount of contrast agent required for optimal PIV analysis. In vitro models at an imaging depth of 10 cm suggested the feasibility of abdominal echoPIV with HFR-CEUS [154]. The objective of this study was to investigate the feasibility of echoPIV to visualize blood flow in the human abdominal aorta by using phase-contrast MRI as a reference.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination 1: high-frame-rate CEUS</td>
<td>5 min - Physical examination and blood pressure</td>
</tr>
<tr>
<td>5 min</td>
<td>- Instructions and visualization of distal aorta</td>
</tr>
<tr>
<td>5 min</td>
<td>- HFR control measurement (Verasonics machine)</td>
</tr>
<tr>
<td>5 min</td>
<td>- Pulsed Wave Doppler measurement (Philips machine)</td>
</tr>
<tr>
<td>5 min</td>
<td>- Insertion of venous cannula</td>
</tr>
<tr>
<td>4 x 2-3 min</td>
<td>- US contrast agent injections (0.25, 0.5, 0.75 &amp; 1.5 ml)</td>
</tr>
<tr>
<td></td>
<td>- HFR-CEUS measurements (Verasonics machine)</td>
</tr>
<tr>
<td>Examination 2: phase-contrast MRI</td>
<td>10 min - Instructions and scanning preparations</td>
</tr>
<tr>
<td>1 hour</td>
<td>- PC-MR scanning</td>
</tr>
</tbody>
</table>

Table 1. Overview of activities for each study participant.

Materials and Methods

This prospective within-subject exploratory study evaluated 15 healthy participants. US and MRI were performed in all participants in February and March 2017, with participants at rest in the supine position. Inclusion criteria were as follows: age of 18–35 years and body mass index of 25 kg/m² or less. Exclusion criteria were as follows: hypersensitivity to the excipients in the US contrast agent (SonoVue; Bracco, Milan, Italy), known history of cardiorespiratory diseases, uncontrolled systemic hypertension, pregnancy, and standard MRI exclusion criteria. Volunteers who met the entry criteria were included in the study after they provided written informed consent. This study was conducted in accordance with Good Clinical Practice guidelines and was approved by an authorized institutional review board in the Netherlands (NL58025.078.16).
**HFR CE echoPIV**

EchoPIV was performed with a fully programmable Vantage 256 US machine (Verasonics, Kirkland, Wash) with a curvilinear array abdominal probe (C5–2; ATL, Bothell, Wash). Before US, physical examination was performed and blood flow velocity in the distal abdominal aorta was measured with pulsed wave Doppler imaging by using an Epiq 7 US machine (Philips Healthcare, Best, the Netherlands).

A four-member research team performed the echoPIV measurements. The aortic bifurcation was visualized in a coronal oblique view by an experienced vascular technologist. The Vantage 256 US machine was controlled by a researcher (J.V.). Contrast agent was injected by a physician with experience in CEUS examinations (P.T.). The Epiq 7 US machine was also used by a researcher (S.E.) for visual contrast monitoring in the left superficial femoral artery. A stable concentration of contrast agent was used for starting the HFR-CEUS measurements, and subsequent injections were given only after substantial washout of the agent. For each measurement, images were captured for 2.5 seconds at 1000 frames per second using a three-angled diverging wave acquisition scheme. First, HFR measurement without contrast agent administration was performed. After this measurement, four incremental contrast agent doses were administered to each participant (0.25, 0.5, 0.75, and 1.5 mL) to investigate the optimal dose for PIV analysis. An overview of the measurement scheme is given in Table 1.

**Mechanical Index**

Before the study, hydrophone pressure measurements were performed to guarantee that pressures in the ultrasound beam field were within safety limits [178] with the transducer at maximum transmitter voltage. Thereafter, transmitting voltage was set to 60% during the first participant measurements. Four measurement sessions were planned, with three or four participants per session. In each subsequent measurement session, the transmitter voltage was further reduced to investigate image contrast and microbubble behavior. The average mechanical index at a depth of 3–5 cm (depth of abdominal aorta) was calculated for each transmitter volt-age used. By following this regimen, measurements were performed at mechanical indexes of 0.09, 0.06, 0.03, and 0.01 (Table 2).

**Data Analysis**

EchoPIV data were processed offline. Singular value decom-position–based clutter suppression was applied to each of the three transmit angles individually [39]. PIV analysis was performed by means of blockwise cross correlation between like-angled transmissions in each image pair by using a modified version of the open-source software PIVlab (V1.41; W. Thielicke) [180]. The mean of the three resulting correlation maps was used for displacement estimation. A four-iteration cross-correlation approach was used,
with a final block size of 7 3 6 mm and 75% overlap. A 15-frame temporal moving average filter and 5 3 3 Gaussian spatial filter were applied for smoothing of the obtained velocity data. An extensive comparison of contrast agent doses, mechanical indexes, US acquisition schemes, and postprocessing methods that were used in this study is reported elsewhere [181].

**Phase-contrast MRI**

All participants underwent phase-contrast 3.0-T MRI (Ingenia; Philips Healthcare) by using a phased-array torso coil within 1 month before or after echoPIV measurements. Multisection two-dimensional survey acquisitions were obtained to localize the distal aorta and iliac arteries. Subsequently, a three-dimensional acquisition was performed with free-breathing retrospective vectorcardiography-gated gradient-echo and echo planar imaging readout (repetition time msec/echo time msec, 8.9/4.6; echo planar imaging factor, 5; flip angle, 10°). Standard four-point three-directional velocity encoding was used with Venc (maximum velocity encoding) of 150 cm/sec [182]. The acquisition volume captured the aortoiliac bifurcation, including renal and external iliac arteries, with 29 reconstructed 2-mm-thick sections, resulting in a voxel size of 1.8 3 1.8 3 2.0 mm. The cardiac cycle was reconstructed into 30 phases. True temporal resolution was 35.6 msec (i.e., 4 3 the repetition time).

**Comparison of EchoPIV and Phase-contrast MRI**

Quantitative comparisons of echoPIV and phase-contrast MRI velocity data were performed. For image registration, an in-house software package (MASS) was used to visualize the three-dimensional phase-contrast MRI velocity data in manually selected planes that showed anatomic dimensions similar to the echoPIV images. Qualitative comparison of the velocity images was performed.

To extract velocity profiles, the phase-contrast MRI data were imported into Tecplot 360 EX (2016 R1; Tecplot, Bellevue, Wash), and a plane was selected by using the previously mentioned method. Further processing and comparison of the data were performed by using Matlab (R2016a; Math-Works, Natick, Mass).

Temporal velocity profiles were extracted from both data sets in five locations on the centerline of the aorta at 1-cm intervals proximal to the bifurcation apex (Figure 1). The time axis of the phase-contrast MRI data was matched to the echoPIV time axis. Spatial velocity profiles were extracted perpendicular to the centerline of the aorta in five locations 1–3 cm proximal to the bifurcation apex.

Cosine similarity between the shape of the temporal and spatial velocity profiles of both data sets was used as a similarity index and was calculated as follows:
\[
(V_{\text{echoPIV}}, V_{\text{MRI}}) \left/ |V_{\text{echoPIV}}||V_{\text{MRI}}| \right. \\
\]

where \((V_{\text{echoPIV}}, V_{\text{MRI}})\) denotes the inner vector product and \(|V_{\text{echoPIV}}||V_{\text{MRI}}|\) is the vector length. Similarity index can range from 21 to 1, where a value of 1 means two curves are colinear. Difference in peak velocity was calculated relative to the phase-contrast MRI data. Bland-Altman analysis was performed for the temporal peak velocities.

Figure 1: Overview of the measurement and registration method for the US particle image velocimetry (echoPIV) and phase-contrast MRI (PC-MRI) data. Probe locations of temporal velocity profiles (○) and spatial velocity profiles (lines) are shown in red for PC MRI data and in blue for echoPIV data. HFR-CEUS = high-frame-rate contrast-enhanced US.

<table>
<thead>
<tr>
<th>MI</th>
<th># participants</th>
<th>Bubble destruction</th>
<th>Adequate contrast signal for PIV analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HFR-CEUS images</td>
<td>Conventional US images</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Abdominal aorta)</td>
<td>(superficial femoral artery)</td>
</tr>
<tr>
<td>0.09</td>
<td>4</td>
<td>Significant destruction</td>
<td>Significant destruction</td>
</tr>
<tr>
<td>0.06</td>
<td>3</td>
<td>Significant destruction</td>
<td>Significant destruction</td>
</tr>
<tr>
<td>0.03</td>
<td>4</td>
<td>Some loss of signal</td>
<td>No visible destruction</td>
</tr>
<tr>
<td>0.01</td>
<td>4</td>
<td>No visible destruction</td>
<td>No visible destruction</td>
</tr>
</tbody>
</table>

Table 2. Overview of Mechanical Index and Observations Concerning Bubble Destruction. The velocity data of the participants measured with mechanical indices of 0.03 and 0.01 were used for comparison between US PIV and phase-contrast MRI. CE = contrast enhanced, HFR = high frame rate, PIV = particle image velocimetry.
Results

Fifteen participants (six men) were included; their median age was 23 years (range, 18–34 years), and their median body mass index was 20.3 kg/m² (range, 17.3–24.9 kg/m²). Contrast agent injections and HFR-CEUS measurements were successful in all participants. Adequate echoPIV results (in terms of cross correlation) were achieved in all participants for all contrast agent doses. Without the US contrast agent, insufficient signal for PIV analysis remained after clutter suppression.

Figure 2: Streamline representation of blood flow velocities during early diastole in participant 7. Similar flow patterns can be observed in both data sets, including a slow (counterclockwise) recirculation zone near the origin of the left common iliac artery. This recirculation zone occurred during a longer time period in the phase-contrast MRI (PC-MR) data (five of 30 phases) than in the US particle image velocimetry (echoPIV) data (10–15 msec). Dashed lines show estimated delineation of the vessel wall.
Figure 3: Temporal velocity profiles in eight participants. Shaded areas represent the range of measured velocities in the five probed locations. Dif_{peak} = difference in peak velocity relative to phase-contrast MRI data, SI = similarity index. • Participants in whom substantial backflow was found in the US particle image velocimetry data but not in the phase-contrast MRI data. ** Participant in whom no substantial backflow was found with either modality.

**Mechanical Index**

Mechanical indexes of 0.09 and 0.06 showed substantial destruction of contrast agent microbubbles in the abdominal aorta during echoPIV (Table 2). This resulted in contrast agent signals that were inadequate for PIV analysis during diastole. Bubble concentration was replenished during systole by new microbubbles entering the field of view. Contrast agent signal also decreased in the superficial femoral artery at the exact time of the HFR-CEUS measurements (Movie 2 [online]). With a mechanical index of 0.03, some bubble destruction was visible in the HFR-CEUS recordings, with no substantial signal decrease in the superficial femoral artery. Contrast agent signal during diastole was adequate for PIV analysis in these measurements. At a mechanical index of 0.01, no bubble destruction was observed. As a result of contrast agent destruction, only the measurements with mechanical indexes of 0.03 and 0.01, which were performed in eight study participants, were used for comparison of echoPIV and phase-contrast MRI (with 0.25 mL of contrast agent).
Fig 4: Bland–Altman plot of peak velocities in eight participants. Mean absolute difference between US particle image velocimetry (echoPIV) and phase-contrast MRI peak velocities is 24 cm/sec (echoPIV is 4 cm/sec lower). The 95% confidence interval ranges from 226 to 18 cm/sec. The negative mean difference is mainly caused by one outlier in the data (volunteer 8). SD = standard deviation.

Flow assessment
Undisturbed forward blood flow was observed in all eight participants during systole for both modalities. During diastole, retrograde flow was observed with both modalities in all participants except participants 2, 7, and 8. In participants 2 and 8, only the echoPIV data showed backflow during diastole, while phase-contrast MRI data did not. The pulsed wave Doppler measurements agreed with the echoPIV measurements, showing a triphasic flow profile with a clear retrograde flow component. No significant retrograde flow was observed in participant 7 with either modality. In this participant, a period of relative blood stasis occurred during diastole. Flow patterns were similar in both the phase-contrast MRI and echoPIV data, including a recirculation zone near the origin of the left common iliac artery during diastole (Figure 2).

Velocity profiles
Temporal velocity profiles corresponded well between the echoPIV and phase-contrast MRI data sets (figure 3). Mean similarity index was 0.98 (range, 0.96–0.99), and the mean difference in peak velocity was 8.5% (range, 0.09%–29%). Bland–Altman analysis is shown in Figure 4. Similar spatial velocity profiles were also found with both modalities (figure 5). Mean similarity index was 0.99 (range, 0.93–1), and the mean difference in peak velocity was 17.0% (range, 4.6%–32.0%).
Discussion

This study shows that quantification of blood flow in the human abdominal aorta is possible with echoPIV, and velocity profiles and data correspond well with those seen with phase contrast MRI. This first-in-human study has demonstrated that assessment of flow patterns in the abdominal aorta is feasible, which can have major implications for the assessment of prognostic factors of vascular disease, indications for treatment, and clinical follow-up.

A large range of blood flow velocities, including velocities greater than 1 m/sec during systole, and very slow flow rates or blood stasis can be registered. In addition, two-dimensional vector fields of blood flow velocity can be used to evaluate flow disturbances, which is not possible with conventional Doppler imaging.

Analysis of the velocity profiles showed good overall agreement between the echoPIV and phase-contrast MRI data. Both techniques have similar spatial resolution (1.75 x 1.5 mm vector resolution and 2.6-mm US section thickness in echoPIV data versus 1.8 x 1.8 x 2.0 mm voxel size in phase-contrast MRI data), whereas the temporal resolution was 30 times higher for echoPIV (1000 frames per second in real time vs 30 phases per cardiac cycle with interleaved sampling in phase-contrast MRI data). Similar retrograde flow patterns were observed in six of eight participants studied. In participants 2 and 8, retrograde flow was observed in the echoPIV data and pulsed-wave Doppler
measurements but not in the phase-contrast MRI data. This could indicate that flow quantification with echoPIV was more accurate in these participants because of a higher temporal resolution and no averaging of multiple heart cycles. However, the difference in flow patterns could also be explained by differences in body position or physiologic status of the participants during imaging.

Substantial bubble destruction occurred in the HFR-CEUS measurements with a mechanical index greater than or equal to 0.06. This caused a decrease in contrast agent signal that rendered echoPIV results unreliable during diastole. These results were unexpected because no bubble destruction was observed during in vitro testing with use of similar acquisition settings and maximum transmitter voltage (mechanical index’ 0.15) [154]. The reduced bubble stability in vivo could be attributed to several physiologic conditions (temperature, gas exchange, pressure) that were not accounted for in vitro [169], [171], [183]. Image registration was performed by manual extraction of a two-dimensional plane from the phase-contrast MRI data to match the echoPIV data. The US insonification plane was not recorded and could therefore not be recreated in the volumetric phase-contrast MRI data. Neighbouring phase-contrast MRI planes were evaluated, showing clear differences in anatomic dimensions, whereas peak velocities showed differences of less than 10%. Thus, it is reasonable to assume that manual spatial matching of phase-contrast MRI and echoPIV data did not cause large differences in flow velocity.

In the echoPIV data, out-of-plane motion of US contrast agent and local imaging artefacts caused local decreases in correlation values and subsequent errors in the velocity vector fields. These errors were reduced by spatial smoothing, but this also removed details in the vector fields. For echoPIV to become a clinically viable technique, further development is required in terms of ease of use, real-time data visualization, and calculation of derived flow parameters. Furthermore, prospective patient studies with echoPIV, in combination with long-term follow-up, are indicated to investigate the predictive value of these flow parameters.

Conclusion

Quantification of blood flow in the abdominal aorta with echoPIV was performed in humans for the first time, demonstrating the feasibility of the technique. An optimal balance between image contrast and bubble concentration was found in a small cohort of healthy participants. The PIV velocity data showed good overall agreement with corresponding phase-contrast MRI data sets. Although it requires further development and validation, the echoPIV technique has great potential to enable quantitative diagnosis of vascular diseases and follow-up after treatment.
Chapter 6
US Velocimetry in Participants with Aortoiliac Occlusive Disease

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Abstract

**Background:** The accurate quantification of blood flow in aortoiliac arteries is challenging but clinically relevant, because local flow patterns can influence atherosclerotic disease.

**Purpose:** To investigate the feasibility and clinical application of two-dimensional blood flow quantification using high-frame-rate contrast-enhanced US (HFR-CEUS) and particle image velocimetry (PIV), or US velocimetry, in participants with aortoiliac stenosis.

**Method:** In this prospective study, participants with a recently diagnosed aortoiliac stenosis underwent HFR-CEUS measurements of the pre- and poststenotic vessel segments (August 2018 to July 2019). Two-dimensional quantification of blood flow was achieved by performing PIV analysis, which was based on pairwise cross-correlation of the HFR-CEUS images. Visual inspection of the entire data set was performed by five observers to evaluate the ability of the technique to enable adequate visualization of blood flow. The contrast-to-background ratio and average vector correlation were calculated. In two participants who showed flow disturbances, the flow complexity and vorticity were calculated.

**Results:** 35 participants (median age, 67 years; age range, 56–84 years; 22 men) were included. Visual scoring showed that flow quantification was achieved in 41 of 42 locations. In 25 locations, one or multiple issues occurred that limited optimal flow quantification, including loss of correlation during systole (n = 12), shadow regions (n = 8), a short vessel segment in the image plane (n = 7), and loss of contrast during diastole (n = 5). In the remaining 16 locations, optimal quantification was achieved. The contrast-to-background ratio was higher during systole than during diastole (11.0 ± 2.9 vs 6.9 ± 3.4, respectively; p < 0.001), whereas the vector correlation was lower (0.58 ± 0.21 vs 0.47 ± 0.13; p < 0.001). Flow complexity and vorticity were high in regions with disturbed flow.

**Conclusion:** Blood flow quantification with US velocimetry is feasible in patients with an aortoiliac stenosis, but several challenges remain for implementation into clinical practice.

Introduction

Time-resolved quantification of blood flow in diseased aortoiliac regions is challenging because of complex flow patterns near the aortic bifurcation and around stenosis. These flow patterns could be used to improve the assessment of stenosis severity and predict disease progression. For example, blood flow patterns have been correlated with the development and progression of atherosclerotic plaques. Specifically, lesions are more likely to form in areas of low wall shear stress [144], [184], which induces major changes in endothelial cells, making the vessel wall more prone to atherosclerosis [47], [49].
The peak systolic velocity ratio obtained with duplex US is traditionally applied to quantify the severity of a stenosis. This parameter shows mixed results compared with the reference standard, the invasively measured pressure gradient over the stenosis [67], [185]. This discrepancy can be explained by the angle-dependency of DUS, only providing a one-dimensional blood flow velocity estimate along the transducer axis, in a complex anatomic region where assumptions about flow direction are often inaccurate [64], [65]. High-frame-rate contrast-enhanced US (HFR-CEUS) combined with particle image velocimetry (PIV), or US velocimetry (echoPIV), enables two-dimensional angle-independent blood flow quantification. EchoPIV could be used to improve and expand the evaluation of lesion severity and to predict atherosclerotic disease progression. A previous study showed that blood flow quantification in the aortoiliac region with echoPIV is feasible in healthy volunteers [186]. However, US imaging in patients with atherosclerosis is more challenging, due to elongated and calcified arteries. This study aimed to investigate the feasibility and clinical application of two-dimensional blood flow quantification using echoPIV, in participants with aortoiliac stenosis.

Materials and Methods

Study Design
This prospective study was conducted in accordance with Good Clinical Practice guidelines, approved by an institutional review board (NL63077.091.17), and registered with the Netherlands Trial Register (NTR6980). Thirty-five consecutive participants aged over 50 years with intermittent claudication (Rutherford category of 1–3) based on aortoiliac stenosis were included after providing written informed consent. Participants were excluded from the study if the use of contrast microbubbles was contraindicated. Demographic and clinical data were retrieved from electronic health records [187], [188]. HFR-CEUS was performed between August 2018 and July 2019 within 1 month after diagnosis. Contrast-enhanced CT scans (section thickness, 0.4 mm) were obtained as an anatomic reference. Agatston calcium scores were calculated by performing automatic calcium segmentation (Intuition, TeraRecon), with a threshold of 600 HU being used to account for the contrast agent-filled vessel lumen [189].

HFR-CEUS Measurements
HFR-CEUS was performed with a Vantage 256 Research US System (Verasonics, Kirkland, WA), and a curved array transducer (GE C1-6D, General Electric Company, Boston, MA). Prior to HFR-CEUS, blood flow velocities were measured with DUS, using an iU22 US machine (Philips Healthcare, Best, the Netherlands).
At each location, two doses of contrast microbubbles (SonoVue; Bracco, Milan, Italy) were administered (9). Microbubble arrival was monitored by using the Verasonics system, with a live imaging sequence at 100 frames/sec. When a quasi-stable concentration of contrast agent was visually established, two HFR-CEUS measurements were performed with a mechanical index of 0.05 and 0.1, both with a center frequency of 2.2MHz and a pulse length of one cycle. Images were captured for 2.5 seconds at 2000 frames/sec with use of a three-angled diverging wave acquisition scheme (pulse repetition frequency, 6000 Hz). Subsequent injections were given after complete washout of the contrast agent on the live images (2–10 minutes after injection). Four measurements were obtained for each location (0.5-mL and 1-mL contrast, each acquired with mechanical indexes of 0.05 and 0.1). This protocol was derived from a previous study [186].

**Data Analysis**

Data were processed off-line with Matlab (R2019b, MathWorks). Raw HFR-CEUS data were reconstructed into images by using coherent compounding of the three transmit angles, increasing contrast and resolution [174]. Clutter suppression was performed using a singular value decomposition based filter [39]. Rank selection (i.e., the cut-off between tissue, blood, and noise) was performed automatically [181]. PIV analysis was performed by using a custom implementation in Matlab consisting of two iterations with a square block size of 5.6 mm and two iterations of 2.8 mm with 75% overlap, resulting in a 0.69-mm vector resolution. To improve the signal-to-noise ratio, correlation averaging was performed over 20 frames, resulting in 100 velocity fields per second. All velocity data and a selection of HFR-CEUS data were stored in a repository that can be accessed (with author permission) at https://www.doi.org/10.4121/c.5497704.

![Figure 1: Average of 100 high-frame-rate contrast-enhanced US images after postprocessing. To calculate the contrast-to-background ratio, eight regions are selected inside the vessel (orange squares), and one region is selected above and below the vessel as the background (blue squares). CIA = common iliac artery.](image)
**Feasibility Scoring**

Qualitative scoring of all velocity data was performed by means of visual inspection by five investigators with expertise in blood flow imaging research (M.M.P.J.R., vascular surgeon with 18 years of experience; E.G.J., assistant professor; J.V., postdoc-toral researcher; and S.E. and M.V., PhD candidates). For each location, the best measurement was selected and used to assess feasibility, which was classified according to three categories: un-feasible (no meaningful information could be obtained), partial quantification (blood flow was adequately visualized, but this only occurred during part of the heart cycle or in a subregion of the imaged vessel because one or multiple issues occurred), or optimal quantification (without any limiting issues).

Definitions for limiting issues (Table 1) were discussed and agreed on by all authors. The investigators were then trained in the application of these criteria by scoring a separate data set that was discussed afterward. Feasibility scoring was then performed independently as described above. Disagreements were resolved in a consensus meeting, and the final score was used for further analysis. Interobserver agreement on the feasibility categories was calculated by using the intraclass correlation coefficient (SPSS Statistics 27, IBM), which was based on a mean-rating, absolute-agreement, two-way, mixed-effects model [190].

<table>
<thead>
<tr>
<th>Limiting issue</th>
<th>Description</th>
<th># cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of correlation (systole)</td>
<td>High velocities (and in some cases disturbed blood flow or high shear) during systole, causing low cross-correlation values during PIV analysis and subsequent loss of velocity vector accuracy. This issue was selected if areas with multiple vectors occurred with correlation values below 0.2</td>
<td>12/42 (29%)</td>
</tr>
<tr>
<td>Short vessel segment</td>
<td>Elongation of the blood vessels (or other anatomical reasons) causing part of the vessel to be outside the imaged plane. The vessel was scored as short, if the visible length was less than 4 diameters.</td>
<td>8/42 (19%)</td>
</tr>
<tr>
<td>Shadow regions</td>
<td>Calcifications in the imaged atherosclerotic plaque causing darker regions (i.e. “shadows”) in the ultrasound images. This issue was selected if contrast in the shadowed region was too low for adequate flow visualization.</td>
<td>7/42 (16%)</td>
</tr>
<tr>
<td>Loss of contrast (diastole)</td>
<td>Destruction of the contrast microbubbles by the ultrasound, causing a severe loss of contrast in the diastolic phase. This issue was selected if all velocity vectors in the imaged region were lost at the end of diastole.</td>
<td>5/42 (12%)</td>
</tr>
</tbody>
</table>

**Table 1: Issues That Limited Flow Visualization.** Data in parentheses are percentages. In some locations, multiple issues occurred; therefore, the total number is higher than the number of measured locations with partial flow visualization. PIV = particle image velocimetry.
Temporal velocity profiles were acquired at five vector locations along the centerline of the vessel and were used to automatically select systolic and diastolic phases. Two feasibility parameters were then calculated for both phases. First, the contrast-to-background ratio was obtained from the HFR-CEUS data by selecting multiple regions within and outside the imaged vessel (Figure 1) and calculating the ratio of the average contrast intensity in those regions. The average normalized cross-correlation value of the velocity vectors was used as a measure of confidence in the velocity vectors, with scores ranging from 0 to 1. Paired t tests were performed to review the difference between systole and diastole. p < 0.05 was considered indicative of statistically significant difference.

<table>
<thead>
<tr>
<th><strong>Patient &amp; Lesion characteristics</strong></th>
<th><strong>Median (range)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>Age (years)</td>
</tr>
<tr>
<td></td>
<td>67 (56-84)</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td>26.4 (20.9-38.5)</td>
</tr>
<tr>
<td><strong># of cases</strong></td>
<td>Sex (man/woman)</td>
</tr>
<tr>
<td></td>
<td>21/13</td>
</tr>
<tr>
<td></td>
<td>Rutherford category (grade 2/3)¹⁰</td>
</tr>
<tr>
<td><strong>Risk factors (SVS grading system)¹¹</strong></td>
<td>Total (grade 1/2/3)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (2/3/0)</td>
</tr>
<tr>
<td>Smoking</td>
<td>23 (5/13/5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (7/8/0)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>4 (0/3/1)</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>32 (0/0/32)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>5 (5/0/0)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>1 (0/1/0)</td>
</tr>
<tr>
<td><strong>Location of the lesion</strong></td>
<td># of cases</td>
</tr>
<tr>
<td>Aortic bifurcation</td>
<td>3</td>
</tr>
<tr>
<td>Common iliac artery - Left</td>
<td>7</td>
</tr>
<tr>
<td>Common iliac artery - Right</td>
<td>11</td>
</tr>
<tr>
<td>Iliac bifurcation - Left</td>
<td>2</td>
</tr>
<tr>
<td>Iliac bifurcation - Right</td>
<td>0</td>
</tr>
<tr>
<td>External iliac artery - Left</td>
<td>9</td>
</tr>
<tr>
<td>External iliac artery - Right</td>
<td>4</td>
</tr>
<tr>
<td><strong>CTA</strong></td>
<td><strong>Median (range)</strong></td>
</tr>
<tr>
<td>vessel diameter before lesion (mm)</td>
<td>7.2 (4.7-12.8)</td>
</tr>
<tr>
<td>vessel diameter after lesion (mm)</td>
<td>7.8 (3.9-13.0)</td>
</tr>
<tr>
<td>Agatston calcium score¹²</td>
<td>1196 (0-4560)</td>
</tr>
<tr>
<td>Cases with shadows in HFR-CEUS data</td>
<td>1614 (107-4063)</td>
</tr>
<tr>
<td><strong>Cases without shadows in HFR-CEUS data</strong></td>
<td>915 (0-4560)</td>
</tr>
</tbody>
</table>

Table 2. Demographic and clinical data of the 34 patients where HFR-CEUS data was acquired. BMI=body mass index, CTA=contrast-enhanced computed tomography. HFR-CEUS = high-frame-rate contrast-enhanced ultrasound. SVS = Society for Vascular Surgery.

* 36 lesions were measured in 34 patients, because 2 patients had a bilateral lesion.
Flow Parameters

Flow complexity, a measure of multidirectional blood flow [32], [191] and vorticity (ie, curl of the vectors [192]) were calculated in two participants with optimal blood flow quantification who showed a region with disturbed blood flow. Both flow parameters were compared with those from an undisturbed region in the same participant.

Results

Thirty-five participants (22 men; median age, 67 years; age range, 56–84 years) were included (Figure 2, Table 2). HFR-CEUS measurements were obtained in 34 of the 35 participants (in one participant, the stenosis could not be visualized with use of either of the US machines). In eight participants, two separate locations were measured, resulting in 42 locations (Figure 2).

Figure 2: Flow diagram shows the inclusion of study participants and the number of measurement locations. echoPIV = US velocimetry.

Feasibility

The intraclass correlation coefficient was 0.30 (95% CI: 0.21, 0.38). Flow quantification was achieved in 98% of measurements (41 of 42). In 38% (16 of 42 measurements), optimal flow quantification was achieved. In 60% (25 of 42) of measurements, only partial flow quantification was possible because of loss of correlation during systole (n = 12) (Figure 3A, Movie 1 [online]), short vessel segments (n = 7) (Figure 3B), shadow regions (n = 8) (figure 3C-D) and loss of contrast during diastole (n=5) (figure 3E-F). Calcium scores in the locations where shadows occurred were higher than in other cases (Table 2).
Figure 3: US velocimetry images show examples of “partial” flow visualization in four participants. Solid red lines indicate the borders of the vessel, and lines within these borders indicate the flow pattern (arrowheads indicate direction). All US images were obtained by placing the transducer on the patient’s abdomen in the long axis of the corresponding vessel. (A) A stenotic lesion (red arrow) in the external iliac artery (EIA) of a 61-year-old man caused very fast and disturbed flow patterns. These patterns could not be adequately visualized. (B) The left common iliac vein (CIV) crosses the right common iliac artery (CIA) in this 58-year-old man, leaving only short arterial vessel segments in the imaged plane.
(C) Shadows caused by calcifications (yellow and blue arrows) limit contrast intensity, and consequently, the visualization of blood flow in this 69-year-old man. (D) Contrast-enhanced CT images of the same participant in C (coronal image with transverse sections at the location of the red lines) shows calcifications on the anterior side of the left common iliac artery (yellow and blue arrows). (E, F) Images in an 84-year-old woman with a stenosis in the proximal common iliac artery show poststenotic disturbed flow during systole (lateral position at around 210 mm). During diastole, the contrast microbubbles were destroyed, and flow visualization was not possible.
The contrast-to-background ratio was significantly higher during systole than diastole (11.0 ± 2.9 vs 6.9 ± 3.4, respectively; p < 0.001). The lowest contrast-to-background ratio values during diastole correspond to the five locations where loss of contrast was observed (Figure 4). The mean correlation values of the velocity vectors were significantly lower during systole (0.58 ± 0.21 vs 0.47 ± 0.13; p < 0.001), except in those same five locations.

**Flow Parameters**

Flow complexity was higher in regions with disturbed flow (figure 5A, green box). In one participant this difference was most pronounced during systole (figure 5C, right side). Both participants showed a similar increase in vortical flow during systole in both regions, but vorticity was higher in the regions with disturbed flow.

![Figure 4: Contrast-to-background ratio (CBR) and mean vector correlation values during systole (blue) and diastole (green). Boxes indicate upper and lower quartiles, and whiskers indicate highest and lowest values. The five cases categorized as having severe contrast destruction at qualitative scoring are presented as separate markers but were included in the paired t-test. ** = For both parameters, there is a significant difference between systole and diastole. The mean contrast-to-background was lower during diastole (6.9 ± 3.4 [standard deviation] vs 11.0 ± 2.9; p <0.001), and the mean vector correlation value was higher during diastole (0.58 ± 0.21 vs 0.47 ± 0.13; p < 0.001).](image-url)
Discussion

This study showed that US velocimetry, or echoPIV, in the aortoiliac tract was feasible in 98% of locations. Optimal quantification was achieved in 38% of locations, indicating that the technique needs to be optimized. In participants with optimal quantification, disturbed blood flow patterns could be clearly distinguished from undisturbed blood flow patterns in areas without an increased peak systolic velocity that were not identified as problematic areas at duplex US. Partial quantification was achieved in 60% of locations. Here, blood flow could still be visualized, but this was only possible during part of the cardiac cycle or in a subregion of the imaged vessel.

We previously showed a good match between echoPIV and phase-contrast MRI in the aortic bifurcation in healthy volunteers [186]. In the current study, echoPIV flow patterns closely matched the movement of the contrast microbubbles on high-frame-rate contrast-enhanced US (HFR-CEUS) images and are therefore assumed to be valid. In most participants, we were not able to obtain reliable Doppler velocity measurements because the maximum angle of 60° was not achieved or because the direction of the flow could not be accurately estimated. Therefore, these data could not be used as a reference. This also confirms the inherent limitations of Doppler imaging due to its angle dependency.

Without the use of duplex US, a reference standard to compare the blood flow velocities measured with echoPIV is lacking. A suitable alternative would have been to use phase-contrast MRI. However, this was not available at our institution during the study. Despite thorough training, the interobserver agreement was still poor (Intraclass correlation coefficient, 0.30), emphasizing the complexity of this unvalidated quality assessment developed in-house. During the consensus meeting, disagreements were mostly caused by different interpretations of the exact cutoff for each limiting issue. Consensus was achieved more easily for each subsequent case, indicating that the interpretation of the scoring criteria converged. Rescoring would therefore likely improve interobserver agreement, but not the scoring method itself.

Optimal blood flow quantification was not achieved in all imaged vessel segments because of several limiting issues. Some of them, including calcifications and out-of-plane blood flow, affect US imaging in general. Loss of contrast (i.e., microbubble destruction) is a problem that is exacerbated with the use of HFR-CEUS because of the increased exposure of individual microbubbles to ultrasound waves. Severe bubble destruction occurred in five participants, whereas only minor destruction occurred in a previous study in healthy volunteers for whom similar US intensities were used [186]. This could be explained by stagnant blood flow during diastole in patients with atherosclerosis, which does not occur in healthy volunteers. Decreasing the mechanical index further to prevent this destruction would have resulted in an inadequate signal-to-noise ratio. This issue could be addressed by using novel contrast agents that are either more stable during insonification or produce stronger US reflections at a lower mechanical index [193].
Figure 5: (A) US were images obtained by placing the transducer on the patient’s abdomen and imaging the long axis of the corresponding vessel. US velocimetry data were obtained during systole in two participants who had flow disturbances at visual inspection. Solid red lines indicate the borders of the vessel, and lines within these borders indicate the flow pattern (arrowheads indicate direction). Flow parameters were calculated in a region with undisturbed flow (blue boxes) and in a region with disturbed flow (green boxes).
CIA = common iliac artery, EIA = external iliac artery. (B) Temporal velocity profiles acquired at five locations along the centerline of the vessel, showing several heart cycles. Shaded error bars represent the range of measured velocities. (C) Flow complexity and (D) vorticity in the region with undisturbed blood flow (blue) and in the region with disturbed blood flow (green). Left side of A–D: blood flow in the left common iliac artery in a 56-year-old woman. Right side of A–D: blood flow in the left external iliac artery in a 69-year-old man.
In addition, echoPIV requires capturing an entire vessel segment in a single image plane. This is challenging in patients with atherosclerosis, who typically have elongated and curved arteries. Three-dimensional US acquisitions are needed to properly capture these out-of-plane vessels. However, this technique is still in early development [110], [111].

In our study, data processing and subsequent flow quantification were performed off-line, without having flow information as feedback to optimize measurement settings and transducer positioning. Translation of echoPIV to daily clinical practice would greatly benefit from real-time flow quantification capabilities, which would require direct data processing [194]. This could then be used to search for clinically relevant flow features, instead of relying on anatomic features.

Despite these technical limitations, flow disturbances were successfully quantified with use of echoPIV by measuring vector complexity and vorticity. These parameters could be used as an alternative for local wall shear stress values, which currently cannot be calculated accurately with use of echoPIV, and may predict atherosclerotic disease progression. In the future, longitudinal studies with a larger sample size will be needed to show the prognostic value and clinical impact of this technology.

**Conclusion**

Blood flow quantification is feasible by using US velocimetry (echoPIV) in patients with aortoiliac stenosis. Technical challenges—such as microbubble stability, three-dimensional imaging methods, and direct data processing—must be addressed for clinical implementation. Nonetheless, echoPIV already enables acquisition of additional information, including vector complexity and vorticity, that can be used to distinguish disturbed from undisturbed blood flow in regions that are not identified as problematic areas by using duplex US.
Chapter 7
Blood flow quantification with high-frame-rate, contrast–enhanced ultrasound velocimetry in stented aortoiliac arteries: in vivo feasibility

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Majorie van Helvert
Jason Voorneveld
Johan G Bosch
Guillaume Lajoinie
Erik Groot Jebbink
Michel MPJ Reijnen
Michel Versluis

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Abstract

**Introduction:** Local flow patterns influence stent patency, while blood flow quantification in stents is challenging. This study aimed to investigate the feasibility of two-dimensional blood flow quantification using high-frame-rate, contrast-enhanced ultrasound (HFR-CEUS) and particle image velocimetry (PIV), or echoPIV, in patients with aortoiliac stents.

**Method:** HFR-CEUS measurements were performed at 129 locations in 62 patients. Two-dimensional blood flow velocity fields were obtained using echoPIV. Visual inspection was performed by five observers to evaluate feasibility. The contrast-to-background ratio and average vector correlation were calculated and compared between stented and native vessel segments.

**Results:** Flow quantification with echoPIV was feasible in 128 locations (99%) with optimal quantification in 40 locations (31%). Partial quantification was achieved in 88 locations (68%), where one or multiple limiting issues occurred (not related to the stent) including: loss of correlation during systole (57/129); short vessel segments (20/129); loss of contrast during diastole (20/129); and shadow regions (20/129). The contrast-to-background ratio and vector correlation was lower downstream in the imaged vessel, independent of the location of the stent. In conclusion, echoPIV was feasible in stents placed in the aortoiliac region and the stents did not adversely affect the flow tracking.

Introduction

Local arterial blood flow patterns affect atherosclerotic disease progression and may predict the outcome of endovascular treatment. A widely investigated mechanism here is low or oscillatory wall shear stress (WSS), which causes disturbances in endothelial cell growth, leading to the formation of atherosclerotic plaques [47]–[49]. When these plaques lead to disabling symptoms, or patients do not respond to exercise therapy, surgical or endovascular revascularization may be indicated. Endovascular treatment of atherosclerotic lesions in the aorto-iliac tract often involves the use of a stent, particularly in more advanced stages. A single stent suffices in most unilateral cases, but treatment with a configuration of multiple stents is required in more extensive lesions involving the aortic bifurcation. A common method to treat these lesions is the “kissing stent” (KS) technique, where 2 stents are simultaneously deployed in the common iliac arteries, protruding into the aorta (figure 1B). This technique is hampered by geometrical mismatches and subsequent disturbed local flow patterns [14], [57]. A newer technique is the “Covered Endovascular Reconstruction of the Aortic Bifurcation”, or CERAB technique [25], where a funnel-shaped aortic cuff is placed in the aorta and two iliac limbs are subsequently placed in the smaller portion of this cuff, making a tight connection and mimicking the natural bifurcation (figure 1C). A study by Groot Jebbink et al. [58] showed
a beneficial flow profile in vitro in the CERAB inflow region, compared to the KS, which could explain its apparent superior performance.

Unfortunately, patient-specific in vivo mechanisms causing stent re-occlusion often remain unclear, but may also be related to blood flow. Accurate quantification of these blood flow patterns may therefore aid in improving the outcomes of endovascular treatment. However, blood flow quantification in the aortoiliac region is challenging, especially in and around stents. Current clinical workflow includes conventional B-mode ultrasound, combined with pulsed wave Doppler measurement, i.e. Duplex ultrasound (DUS). However, Conventional Doppler ultrasound only provides one-dimensional blood flow velocity estimations along the transducer axis, which are often inaccurate in complex anatomic regions where the direction of the flow cannot be determined reliably by the ultrasonographer [64], [65].

Phase contrast MRI can provide three-dimensional quantification of blood flow patterns in large volumes of interest [95], although it is relatively expensive and time-consuming and therefore less likely to be implemented in a standard clinical workflow for vascular patients. Furthermore, accurate flow quantification is deemed unfeasible inside some types of stents, as they cause disturbances in the magnetic field leading to imaging artefacts [104], [105].

These limitations can be overcome by newer ultrasound techniques, such as high-frame-rate contrast-enhanced US (HFR-CEUS) combined with particle image velocimetry (PIV), or echoPIV. PIV is a flow tracking method that was originally applied in fluid dynamics research where seeded particles are used to scatter light from a thin laser-sheet onto a high-speed camera [41]. This method was first adapted using a clinical ultrasound machine by Kim et al [159]. However, the use of conventional, line-scanning ultrasound
imaging, resulted in underestimation of high velocities [86], [145]. Recent echoPIV methods use plane-wave and diverging-wave ultrasound imaging to obtain several thousands of images per second. This high-frame-rate echoPIV technique has been successfully used to quantify high velocity (~1m/s) blood flow in animal studies of the aorta and the heart [88], [157].

A recent study showed that 2-dimensional blood flow quantification with echoPIV in the aortoiliac tract is feasible in patients with untreated aortoiliac occlusive disease [195]. However, echoPIV in patients with stents is more challenging because of possible attenuation artifacts caused by the metal stent struts. Therefore, this study aimed to investigate the feasibility of 2-dimensional blood flow quantification with echoPIV in and around stented vessel segments of patients with aortoiliac disease.

**Materials and Methods**

Between August 2018 and March 2021, HFR-CEUS measurements were performed on patients with a single stent, or a complex stent configuration (KS or CERAB, Figure 1B-C). Demographic data were retrieved from electronic health records and used as a reference, clinical imaging data was used to determine the location and type of the stents. The study was conducted in accordance with Good Clinical Practice guidelines, approved by an institutional review board (NL63077.091.17) and registered at the Netherlands Trial Register (NTR6980). All patients provided written informed consent prior to enrollment. The measurement protocol and data analysis was similar to an earlier feasibility study in untreated patients with aortoiliac occlusive disease [195].

**HFR-CEUS measurements**

All HFR-CEUS measurements were performed within 2 months after stent placement, using a Vantage 256 US System (Verasonics, Kirkland, WA) connected to a curved array transducer (GE C1-6D, General Electric Company, Boston, MA; 3.4 MHz center frequency, 192 elements, 66 mm elevation focus, 0.35mm pitch). Prior to the HFR-CEUS measurements, the appropriate imaging location was confirmed using DUS on an iU22 US machine (Philips Healthcare, Best, the Netherlands).

In patients treated with a single stent, measurements were performed in one or two locations, to cover both the inflow and outflow tract of the stent (figure 1A). Four measurements per location were performed, with a mechanical index (MI) of 0.05 and 0.1 and acquired after a 0.5 mL or 1.0 mL Sonovue (Bracco Imaging, Milan, Italy) microbubble contrast injection. In patients treated with a single stent, measurements were performed in one or two locations, to cover both the inflow and outflow tract of the stent (figure 1A). Four measurements per location were performed, with a mechanical index (MI) of 0.05 and 0.1 and acquired after a 0.5 mL or 1.0 mL Sonovue (Bracco Imaging, Milan, Italy) microbubble contrast injection. In patients treated with a KS or CERAB configuration, three locations were measured: the aortic inflow tract and both iliac outflow tracts (figure 1B-C). In these patients, two measurements per location were performed with both MI’s after the administration of 1.0 mL contrast (due to limitations in total dosage).
Microbubble arrival was monitored with the Verasonics, using a low-frame-rate, three-angled diverging wave Pulse Inversion (PI) sequence, with 6 acquisitions per frame and 100 frames per second (fps). Each HFR-CEUS recording started when the initial contrast bolus had passed and a quasi-stable concentration of contrast signal was established, 20 to 60 seconds after contrast injection. Images were then acquired for 2.5 seconds using a three-angled acquisition scheme with a single diverging wave for each angle (-18°, 0° and +18°), a 6000 Hz pulse repetition frequency, 2.23 MHz center frequency and 1 cycle pulse length. Contrast-specific pulse sequences were not applied.

Data analysis
All data was processed offline using Matlab (R2019b; MathWorks, Natick, MA). All HFR-CEUS images were reconstructed using the Verasonics reconstruction software (version 4.0), by coherent compounding of the beamformed in-phase quadrature (IQ) data obtained with the three transmit angles, resulting in a total frame rate of 2000 fps. Clutter suppression was performed on the compounded IQ data, with the region of interest (the aorta and/or iliac artery) cropped out. A singular value decomposition (SVD)-based filter was used [39], [162], with automatic rank selection based on the spatial distribution of blood and tissue signal in the decomposed spatial similarity matrix [40]. In this similarity matrix, distinct subspaces occur where the singular vectors representing tissue and blood signal correlate with itself, but ideally not with each other. A single lower threshold was used without a higher rank cut-off, as bubble signal was also visible in higher order singular values. EchoPIV analysis was performed on the signal envelope, using a custom PIV implementation in Matlab [177], [181]. This implementation calculated the blockwise normalized cross-correlations in the Fourier domain between each pair of image sub-regions (i.e. kernels) in four iterations (two iterations with a square block size of 32 pixels, or 5.6 mm and two iterations of 2.8 mm, with 75% overlap, resulting in a 0.69 mm vector resolution), using a window deformation scheme with linear interpolation. For each iteration, parabola peak fitting was used to estimate the sub-pixel displacement (within a 2x3 pixels window). Between iterations, velocity vectors based on a correlation value <0.1 were assumed to be erroneous and median outlier detection was applied to find additional erroneous vectors. These erroneous vectors were then discarded and subsequently interpolated from surrounding valid vectors. Post-processing after PIV analysis consisted of correlation averaging over 10 frames, finally resulting in 200 velocity fields per second. Spatial and temporal smoothing was then applied to these velocity fields, using a temporal moving average filter (3 ensembles) and a spatial Gaussian filter ($\sigma = 0.5 \times 0.5$ and extent = 3 x 3). All velocity data and a selection of HFR-CEUS data (raw channel data and IQ data) were stored in a repository that can be accessed (with author permission) at: https://www.doi.org/10.4121/c.5615425
Feasibility scoring

All velocity datasets were visually inspected and scored independently by five investigators (SE, MvH, EGJ, JV, MMPJ), with a final score based on consensus, using a previously described methodology [195]. For each location, the best measurement was selected from the different MI and contrast dosages and used to assess feasibility, using three categories:

1. Unfeasible, meaning blood flow could not be visualized.
2. Partial, meaning blood flow was adequately visualized, but only in a sub-region of the imaged vessel or during one part of the heart cycle, due to one or multiple limiting issues.
3. Optimal, meaning blood flow was visualized in the entire imaged blood vessel, during the entire cardiac cycle, without any limiting issues.

Limiting issues included:

1. Loss of correlation during systole, due to high velocities or complex flow, including out-of-plane flow.
2. Short vessel segments in the US image, due to complex 3D anatomy of the arteries.
3. Loss of contrast during diastole, due to destruction of microbubbles as a result of repetitive insonations.
4. Shadow regions, due to calcified atherosclerotic plaques or other attenuating structures, leading to erroneous vectors.

Figure 2: Region selection for the calculation of CBR and normalized average vector correlation. The regions in the stent (white squares) were compared to the regions in the native vessel (black squares). In this example, both the inflow and outflow tract of the stent was used for the comparison. Shadow regions (red arrows) were disregarded for this analysis. For the CBR, the ratio of the signal intensity between the contrast regions (white and black squares) and tissue regions (blue squares) was calculated. Vessel wall delineation (red lines) and stent location (white lines) was manually drawn, based on the unfiltered HFR-CEUS data and other modalities (conventional DUS, angiographic images and PI live view).
**Stented vs native vessel segments**

To quantify the influence of the stents on the visualization of contrast microbubbles and tracking performance of the PIV algorithm, the contrast-to-background ratio (CBR) and vector correlation were calculated for each frame in separate parts of the imaged vessel with and without a stent, i.e. the stented and native vessel segments (figure 2). Peri-procedural angiography images, DUS images (made with the Phillips iU22) and low-frame-rate PI images (made with the Verasonics) were used to determine the location of the stents on the HFR-CEUS images. CBR and vector correlation were then calculated in all measurements where (1), the proximal or distal stent edge could be precisely determined and (2), where contrast microbubbles were visible in both the native and stented vessel segments, over a length of more than two vessel diameters, excluding shadow regions with decreased US signal (Figure 2).

To calculate CBR and vector correlation, 4 regions were selected in the native and stented vessel segments, together with 2 tissue regions that were needed for the CBR (figure 2). CBR was then computed in all frames, for the native and stented vessel segments, using:

\[
CBR (dB) = 10 \log_{10} \left( \frac{RMS_{c1-4}^2}{RMS_{t1-2}^2} \right),
\]

with RMS representing the root-mean-square signal strength of the contrast and tissue regions.

Vector correlation was defined as the average of all maximum-cross-correlation values, ranging between 0 and 1, for all image kernels within the selected native and stented vessel regions. Finally, the CBR and vector correlation values were averaged for all frames, and for systolic and diastolic frames separately, to allow the comparison of these two time periods within the cardiac cycle.

**Statistical analysis**

Statistical analysis was performed with IBM SPSS 27 (IBM Corp, Armonk, NY, USA). Categorical variables (in the visual feasibility scoring) are represented as number of cases (%). Continuous variables (in the quantitative comparison of stented and native vessel segments) are given as median (interquartile range, IQR). Interobserver agreement on the feasibility categories was calculated using the intraclass correlation coefficient, based on a mean-rating, absolute-agreement, two-way mixed-effects model [190]. Differences in CBR and vector correlation were tested with a paired t-test for normally distributed data and a Wilcoxon signed rank test for data that was not normally distributed (including all cases where one of the two compared variables was not normally distributed). A p-value < 0.05 was considered statistically significant.
Results

Eighty-five patients were enrolled, of which 23 were excluded for several reasons: 9 planned HFR-CEUS measurements were cancelled, in 3 cases due to COVID-19 restrictions. In 7 patients, placement of an intravenous canula was not successful after one single attempt. In another 7 patients, the inflow and outflow region of the stent could not be located on either US machines and therefore no HFR-CEUS measurements were attempted (figure 3). HFR-CEUS data was successfully acquired at 129 locations in 62 patients (42 men, median age: 67 years, IQR: 60-76 years, median BMI: 26.2, IQR: 24.2-27.5). This included 42 patients with a single stent (figure 1A) and 20 patients with a complex stent configuration (KS in 8 cases, CERAB in 12 cases, Figure 1B-C).

Figure 3: Overview of the number of patient included and HFR-CEUS measurements performed. HFR-CEUS = high-frame-rate, contrast-enhanced ultrasound, COVID-19 = coronavirus disease 2019, IV = intravenous (canula).
Figure 4: Examples of limiting issues resulting in partial flow visualization. Vessel walls were manually drawn with red lines (arteries) or blue lines (veins), stents are represented with white lines. A: loss of correlation during systole (red arrow), in the origin of the right common iliac artery (CIA), near the proximal edge of the stent (supplemental video 1). B: A short segment of the Left CIA with the left common iliac vein (CIV) also in the image (supplemental video 2). C: loss of contrast during diastole in the left external iliac artery (EIA). A systolic frame is shown on the left, a diastolic frame is shown on the right (supplemental video 3). D: A shadow region (red arrow) caused by bowel gas (yellow arrow), just distal to the Iliac bifurcation (supplemental video 4).
Feasibility scoring
Two-dimensional flow visualization with echoPIV was feasible in 128 of 129 locations (99%) where HFR-CEUS data was acquired. Optimal visualization was achieved in 40 locations (31%) and partial visualization in 88 locations (68%). These vector fields suffered from loss of correlation during systole in 57 of 129 cases, or 45% (figure 4A, supplemental video 1). Short vessel segments (figure 4B, supplemental video 2), loss of contrast during diastole (figure 4C, supplemental video 3) and shadow regions (figure 4D, supplemental video 4) all occurred in 20 of 129 cases, or 16%. The intraclass correlation coefficient (for the agreement between observers in the feasibility scoring) was 0.664 (95% CI: 0.611, 0.717).

Stented vs native vessel segments
25 inflow regions and 42 outflow regions met the criteria for comparison of the stented vessel segment with the native (non-stented) segment (figure 2). These imaged regions contained several types of stents, including 11 Advanta V12 stents (Maquet Getinge group, Rastatt, Germany), 16 BEgrafts (Bentley InnoMed GmbH, Hechingen, Germany), 10 Everflex stents (Medtronic plc, Minneapolis, MN, USA), 16 Express stents (Boston Scientific Corp, Marlborough, MA, USA), 2 SMART stents (Cordis, Hialeah, FL, USA) and 12 Viabahn stentgrafts (W.L. Gore and Associates, Flagstaff, AZ, USA). An overview of stent characteristics and the number of stents in both regions is shown in Table 1.

At the inflow regions, the CBR and vector correlation in the stented vessel segments was lower, compared to the native vessel segments (Table 2, Figure 5 A&C). For the outflow regions, the CBR and vector correlation was higher in the stented vessel segments, compared to the native vessel segments (Table 3, Figure 5 B&D). These differences were statistically significant in both the inflow and outflow regions, for all frames and both the systolic and diastolic phase of the cardiac cycle (p < 0.05), except for the average vector correlation in all frames at the inflow regions (p = 0.059, figure 5C).

In both the inflow and outflow regions, CBR was significantly higher during the systolic phase compared to the diastolic phase, independent of the stent location (Table 2 and 3, Figure 5 A-B). Conversely, vector correlation was significantly lower during the systolic phase, compared to the diastolic phase (Table 2 and 3, Figure 5 C-D).
Table 1. Overview of stent characteristics, including the number of imaged stents in the inflow and outflow regions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>material</th>
<th>cover</th>
<th># cases in inflow region</th>
<th># cases in outflow region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanta V12</td>
<td>Maquet</td>
<td>Stainless steel</td>
<td>PTFE</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>BEgraft</td>
<td>Bentley</td>
<td>Cobal-chrome</td>
<td>PTFE</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Everflex</td>
<td>Medtronic</td>
<td>nitinol</td>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Express</td>
<td>Boston scientific</td>
<td>Stainless steel</td>
<td></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SMART</td>
<td>Cordis</td>
<td>nitinol</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Viabahn</td>
<td>Gore</td>
<td>nitinol</td>
<td>PTFE</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

Nitinol = nickel aluminum alloy, PTFE = Polytetrafluoroethylene

Table 2. CBR and average vector correlation at the stent inflow regions. Data is represented as median (interquartile range) and organized similar to figure 5 (where the inflow regions are shown in panel A & C), with the stented vessel segment on the right side.

<table>
<thead>
<tr>
<th>inflow</th>
<th>Native vessel segment</th>
<th>Stented vessel segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBR</td>
<td>All frames</td>
<td>All frames</td>
</tr>
<tr>
<td></td>
<td>Systole</td>
<td>Systole</td>
</tr>
<tr>
<td></td>
<td>Diastole</td>
<td>Diastole</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.1 (4.7)</td>
<td>7.8 (5.6)</td>
</tr>
<tr>
<td></td>
<td>13.1 (4.1)</td>
<td>10.2 (3.7)</td>
</tr>
<tr>
<td></td>
<td>8.4 (5.9)</td>
<td>5.6 (5.9)</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.77 (0.17)</td>
<td>0.71 (0.19)</td>
</tr>
<tr>
<td></td>
<td>0.57 (0.36)</td>
<td>0.44 (0.19)</td>
</tr>
<tr>
<td></td>
<td>0.82 (0.21)</td>
<td>0.80 (0.35)</td>
</tr>
</tbody>
</table>

CBR = contrast-to-background ratio

Table 3. CBR and average vector correlation at the stent outflow regions. Data is represented as median (interquartile range) and organized similar to figure 5 (where the outflow regions are shown in panel B & D), with the stented vessel segment on the left side.

<table>
<thead>
<tr>
<th>outflow</th>
<th>Stented vessel segment</th>
<th>Native vessel segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBR</td>
<td>All frames</td>
<td>All frames</td>
</tr>
<tr>
<td></td>
<td>Systole</td>
<td>Systole</td>
</tr>
<tr>
<td></td>
<td>Diastole</td>
<td>Diastole</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5 (5.4)</td>
<td>6.6 (4.8)</td>
</tr>
<tr>
<td></td>
<td>13.0 (6.8)</td>
<td>10.0 (5.3)</td>
</tr>
<tr>
<td></td>
<td>5.6 (7.8)</td>
<td>4.8 (4.4)</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.69 (0.22)</td>
<td>0.64 (0.22)</td>
</tr>
<tr>
<td></td>
<td>0.42 (0.20)</td>
<td>0.41 (0.12)</td>
</tr>
<tr>
<td></td>
<td>0.79 (0.20)</td>
<td>0.63 (0.46)</td>
</tr>
</tbody>
</table>

CBR = contrast-to-background ratio
Discussion

This study showed that 2-dimensional flow visualization is possible inside stented aortoiliac arteries. Specifically, echoPIV measurements were feasible in 99% of the measured locations, in and around stents in the aortoiliac region. Optimal quantification was achieved in 31% of all locations. In these locations, blood flow was visualized in the entire imaged vessel, including the stented vessel segment, during the whole cardiac cycle. This data could be used to accurately quantify local arterial flow disturbances, using echoPIV-derived flow parameters such as vector complexity and vorticity. In an earlier study, it was already shown that these flow parameters can be used to distinguish disturbed blood flow from undisturbed blood flow in areas without an increased peak systolic velocity that would typically not be identified as problematic with DUS [195]. Another study by Hansen et al. [196] showed that these parameters are better suited for the assessment of stenosis severity than the conventional peak systolic velocity ratio obtained with DUS.

If algorithms for accurate vessel wall detection and near-wall flow quantification can be further improved, these flow fields can also be used to calculate parameters such as WSS or the oscillating shear index, that are closely related to the development and progression of atherosclerotic lesions [47], [48], [52], [53]. In the future, echoPIV could be used as a, low-cost, easy-to-use tool for flow quantification inside stents in the current workflow of vascular clinics. For example, local flow patterns may be evaluated during endovascular procedures, to improve the stent positioning or stent configuration in real time. Alternatively, local flow disturbances may be monitored during patient follow-up with echoPIV, to allow early re-intervention preventing stent occlusions.

In 68% of the locations, partial flow quantification was achieved. Here, blood flow could still be adequately visualized, but only in a subregion of the imaged vessel or during a part of the cardiac cycle. In these locations, several limiting issues occurred that prevented optimal quantification. Some of these issues, such as calcifications and out-of-plane blood flow, affect US imaging in general, while others apply only to echoPIV. However, it is important to note that none of these limiting issues were related to the stent, as the same limiting issues were already found and discussed in our study in untreated patients with aortoiliac occlusive disease [195]. These limiting issues can be overcome with further improvement of the echoPIV technique, including microbubble stability, three-dimensional imaging and near instantaneous data processing. This would allow visualization of the full flow field in complex three-dimensional anatomies, with direct feedback to optimize imaging location and acquisition settings, further increasing the robustness of the echoPIV technique. If these improved echoPIV algorithms are implemented in the next generation of commercially available ultrasound machines, the technique could be easily adopted into the clinical workflow.
Figure 5: CBR and mean vector correlation values during all frames (blue), systole (red) and diastole (yellow). Boxes indicate upper and lower quartiles, whiskers indicate highest and lowest values. The native vessel segment is shown on the left for the inflow regions, while it is shown on the right for the outflow regions, similar to their location on the US images. * = statistically significant difference ($p < 0.05$). A: CBR in the 25 selected inflow regions. B: CBR in the 42 selected outflow regions. C: correlation in the 25 selected inflow regions. D: correlation in the 42 selected outflow regions.

The CBR and vector correlation was higher in the native vessel segments at the inflow region when compared to the stented vessel segments, but lower at the outflow region. In other words, the CBR and vector correlation was consistently higher upstream in the imaged vessel, regardless of the position of the stent. This can be explained by destruction of the microbubbles while they pass through the image plane, with increasing exposure to US leading to a higher fraction of destroyed bubbles. This results in a downstream decrease in CBR and subsequent decrease in correlation. Voorneveld et al. [181] used this downstream contrast difference to calculate the specific microbubble destruction rate for multiple US acquisition settings.
In conclusion, while differences in CBR and vector correlation were observed in downstream distance, no additional influence of the stent on these quality parameters was found. These results hold in a variety of stent types, made from different materials, that were investigated in this study. This makes flow quantification inside stents with echoPIV superior to 4D flow MRI, where several stent types, such as stainless steel stents, cause artefacts that render flow quantification unreliable [105].

In a previous in vitro study on the performance of echoPIV in a stented carotid artery phantom, a decreased image contrast was found in the stented region, as opposed to our study (Hoving et al. 2021). However, this did not have a negative effect on the velocity estimation.

The lower CBR that occurred during the diastolic phase, as compared to the systolic phase, can also be explained by microbubble destruction. Especially in cases with stagnant flow, CBR gradually decreased during each heart cycle, until no contrast signal remained at the end of the diastolic phase. The concentration of microbubbles is then replenished during systole by new blood coming into the image. Conversely, correlation values during diastole however were consistently higher, which can be explained by the lower diastolic blood flow velocities that generally improve PIV tracking performance.

To reduce bubble destruction and thereby improve the ability of echoPIV to visualize stagnant flow, Image settings should be optimized in the future for each patient specifically. Alternatively, advanced imaging sequences could be developed that use a lower MI or framerate during diastole.

Limitations

The feasibility score in this study was based on classification by several observers, which is inherently subjective. However, all observers are experienced in blood flow imaging and were trained on the application of the scoring criteria. The ICC score was 0.664, indicating moderate agreement between the observers. Most disagreements occurred in cases where a minor “borderline” limiting issue occurred, that some of the observers scored as relevant while others did not.

Visual inspection of the velocity data showed that the PIV-calculated flow patterns in this study closely matched the movement of contrast microbubbles on the HFR-CEUS images. In a previous study, we showed a good match between echoPIV and phase-contrast MRI findings in healthy subjects [186]. Therefore, the flow quantification in this study is assumed to be valid. However, reference measurements to validate the quantified blood flow velocities, were not performed. Doppler ultrasound measurements were attempted, but in many cases were not reliable, because the maximum angle of 60° was not achieved or because the direction of the flow could not be reliably determined. Phase-contrast MRI could also be used as reference, but this has several limitations such as averaging of the velocity data over multiple heartbeats and unreliable velocities inside stents. Furthermore, this technique was not available in our institution at the time of this study.
Conclusion

Two-dimensional blood flow visualization with echoPIV was feasible inside a variety of stents in the aortoiliac region. Furthermore, these stents did not cause a decrease in image quality or tracking performance of the echoPIV algorithm. Several limiting issues, unrelated to the stent, remain that prevent optimal flow quantification. However, with further development of the technique, echoPIV could be used in a clinical setting to improve the outcomes of endovascular treatment in the aortoiliac tract.
Chapter 8
General discussion and future perspective
Although the pathologic mechanisms behind aortoiliac occlusive disease (AIOD) are strongly influenced by local blood flow patterns, these are not important factors in current clinical practice for the diagnosis and treatment of the disease. However, accurate quantification of these blood flow patterns could improve the diagnosis and treatment of patients with AIOD. Unfortunately, real-time blood flow quantification is extremely challenging.

A promising technique for this application is echoPIV, that combines high-frame-rate contrast-enhanced US (HFR-CEUS) with particle image velocimetry (PIV). The main goal of this thesis was to investigate the feasibility and clinical application of echoPIV, in order to facilitate the translation of this technique from the bench to the patient’s bedside.

**Summary of findings**

In chapter 2, the link between local blood flow patterns and AIOD was described in detail. Blood flow patterns influence the development and progression of atherosclerosis and could therefore play an important role in the clinical monitoring of the disease. Furthermore, local blood flow influences the patency of stents. Detailed information on flow disturbances could be used to improve endovascular treatment and post-operative care.

Current diagnostic techniques, such as conventional Duplex Ultrasound and CT-angiography, were also discussed in chapter 2, including their limitations in the evaluation of local hemodynamic factors. Here, it became clear that there is a need for an improved modality to estimate lesion severity and to predict disease progression and stent patency, based on measured local blood flow parameters. This chapter then provided an extensive overview of novel blood flow quantification techniques, including ultrasound-based methods such as echoPIV, 4D flow MRI, and computational fluid dynamics (CFD).

Novel techniques, that allow detailed (and real-time) blood flow quantification in vivo, are not yet widely available in daily clinical practice and therefore cannot be used to guide patient-specific treatment decisions. However, valuable general insights into blood flow related issues can already be obtained in vitro. In chapter 3, local blood flow patterns were quantified using laserPIV, in two simplified models of the aortic bifurcation, with and without a Covered Endovascular Reconstruction of the Aortic Bifurcation (CERAB). The goal of this study was to investigate a common clinical problem: the re-occlusion of a stent due to compromised distal outflow.

Our study showed that local wall shear stress (WSS) decreases more in the CERAB configuration, compared to the control model, when a distal stenosis is added to the flow setup. These findings could explain the relation between distal run-off and stent re-occlusion, which is a valuable insight for the development of novel endovascular devices and treatment protocols.
The dot on the horizon, however, is accurate quantification of blood flow in patients. To introduce new flow quantification techniques into the hospital, the feasibility of these techniques must be evaluated and their added value must be demonstrated. In chapters 4 and 5, the feasibility of echoPIV was investigated in healthy volunteers. The feasibility and clinical application of echoPIV in patients with aortoiliac disease, with and without stents, was investigated in chapters 6 and 7.

Chapter 4 includes a parameter study into different acquisition and processing settings. This study resulted in several important findings that were then introduced in the optimized imaging protocols for echoPIV feasibility testing in patients (chapter 6 and 7). First, microbubble destruction occurred in all but the lowest ultrasound pressure levels. This means that there is a delicate balance between contrast signal loss and a sufficiently high signal-to-noise ratio.

Second, clutter filtering based on singular value decomposition (SVD) turned out to be more effective at suppressing tissue signal than non-linear contrast imaging using amplitude modulation. This matches practical experiences during the patient study where another nonlinear contrast acquisition scheme, pulse inversion (PI), was used to monitor contrast agent arrival. PI resulted in improved image quality after low-pass filtering, compared to high-pass filtering. Moreover, the microbubbles were only visible in fast blood flow conditions during systole. Here, we hypothesized that there was little harmonic frequency content in the microbubble signal, while fast moving microbubbles led to an incomplete cancellation of both acquisitions in the PI imaging. Analysis of the frequency content in the ultrasound signals originating from the microbubbles indeed revealed very low harmonic content.

In conclusion, analysis of the spatiotemporal characteristics of the contrast signal for our study turned out to be more effective than the detection of the nonlinear harmonic content. However, the arrival of novel contrast agents and more broadband CMUT transducers in the not-so-distant future, see chapter 6, may well change our present understanding.

In chapter 5, echoPIV-derived flow velocities from eight volunteers were compared with a reference: 4D flow MRI. Overall, the measured peak velocities during systole and the temporal profile as a whole were very similar in both techniques, indicating the validity of echoPIV. Specifically, in one volunteer that showed stagnant blood flow during diastole, both techniques showed a small and short lived recirculation at the exact same moment (end of systole) and location (origin of the left common iliac artery). This provided additional confidence in the ability of echoPIV to quantify detailed flow patterns.
In two other volunteers, echoPIV showed backward flow during diastole and 4D flow MRI did not. While these measurements were performed on different days, which could have explained the discrepancy, we believe that the difference is a result of the averaging of multiple heart cycles in 4D flow MRI and that real-time echoPIV displays the true velocities. With an optimized acquisition sequence and successful validation of the echoPIV method in healthy volunteers, the technique was ready for application in patients. In chapter 6, the feasibility and clinical application of echoPIV in patients with aortoiliac disease was investigated. This study shows that flow quantification with echoPIV was feasible in 99% of the patients, although some limiting issues occur in over half of the patients that lead to an incomplete visualization, i.e. only during part of the cardiac cycle, or only in parts of the imaged vessel segment.

Limiting cases did not occur in healthy volunteers and are therefore related to the anatomy and physiology of patients with AIOD. For example, arterial calcifications (or other structures such as bowel gas) attenuate ultrasound, leading to a drop in US pressure and causing so-called shadow regions. Furthermore, short vessel segments with out-of-plane blood flow occurred due to a more complex anatomy in patients, as well as a decorrelation in the PIV tracking algorithm in areas with high velocities. Finally, microbubble destruction caused by prolonged exposure to ultrasound occurred in patients with stagnant blood flow during diastole. Shadow artefacts and out-of-plane blood flow are also common limiting cases in conventional Duplex ultrasound. However, microbubble destruction applies specifically to HFR-CEUS and is a target challenge to be solved in the future for improved applicability of the echoPIV technique in patients with AIOD (future perspectives section).

Despite these limitations, echoPIV-derived flow parameters (vector complexity and vorticity) could be used to successfully identify blood flow disturbances in regions with normal peak systolic velocity, that would not be identified as problematic using conventional duplex US.

In chapter 7, the robustness of echoPIV was investigated, by testing the feasibility of flow quantification inside stents placed in the aortoiliac region. This study showed that echoPIV is feasible near and inside a wide variety of commonly used stents. Furthermore, these stents do not degrade the image quality or tracking performance of the echoPIV algorithm. On the contrary, several types of stents interfere with the magnetic field in 4D flow MRI, resulting in imaging artefacts and inaccurate flow quantification. This demonstrated benefit of echoPIV, together with the cost-effectiveness and ease-of-use of ultrasound, shows the potential for implementation into a clinical workflow, despite the need for administration of a contrast agent. This could drastically improve the future outcomes of endovascular treatment in the aortoiliac tract.
**General discussion**

The goal of this thesis was to move echoPIV forward from the lab into the clinic, i.e. from the bench to the bedside. This process generally starts with the development of a technique, followed by validation and feasibility testing in vitro. For echoPIV, these steps were already taken by several other authors before the start of my PhD.

Several other US-based techniques exist that can be used for two-dimensional quantification of blood flow (see chapter 2). Some of these techniques, such as transverse oscillations and speckle tracking, are already commercially available on select clinical scanners. For these techniques, the road towards clinical implementation is therefore much shorter than for echoPIV. Furthermore, these techniques do not require the intravenous injection of microbubbles, making their adoption into clinical practice much easier. Thus, their use in a large national screening program is feasible, provided that there is an added clinical benefit.

Unfortunately, non-contrast US techniques have a limited imaging depth, either due to the limited width of the transducers or due to a low signal-to-noise ratio. This means that these techniques are not ideal for application in deeper lying vessels, such as the aorta and iliac arteries. Here, echoPIV can be useful, with the contrast microbubbles increasing the signal-to-noise ratio at larger depths. While echoPIV is unsuitable for use in the general population due to the use of intravenous contrast, a wide collection of other clinical applications could drastically improve the diagnosis and treatment of patients with AIOD (see future perspectives). However, before the start of my PhD, echoPIV was still in the early development phase and therefore still had a long way to go on the road from the lab to the daily clinical practice. Several lessons learned while traveling this road are described here.

After the initial development and in vitro validation phase, the next step in the implementation of a new medical imaging modality is in vivo feasibility testing. This is done in healthy volunteers first (chapter 4 and 5) and later in patients (chapter 6 and 7). Before this phase can start, safety testing must be performed and certification must be obtained for use in humans. This can be a complex and sometimes cumbersome task that would deserve a publication in its own right. However, the outcomes cannot be generalized to other applications and they are therefore not useful scientific insights. Even when a technique is proven safe, many other (bureaucratic) hurdles remain before it can be introduced into the hospital. While the new European medical device regulation (MDR) should streamline this process, each hospital still has its own specific procedures to follow.

When testing in volunteers, in vivo validation of an imaging method can be performed with a reference standard (chapter 5) and many different settings can be tested to optimize it for use in humans (chapter 4). The question that arises here is: how long to continue optimizing a technique, before testing it in a clinical setting? While improvements bottom-up are always possible, it is impractical to put major effort into a technique that provides limited added benefit in the clinic. This added benefit can only be shown top-down in a clinical setting, but premature attempts can fail to achieve this goal due to technical limitations that occur in the early development of a technique.
Thus, while the echoPIV technique was still far from perfect, we started investigating its feasibility in a clinical setting. While this exercise led to new insights that provided useful feedback for further development strategies for echoPIV, it also clearly demonstrated the potential benefit of the technique in a clinical setting.

After switching from volunteers to patients, many new challenges await (chapter 6). In our case, patients with AIOD have a higher BMI compared to younger, healthy volunteers, resulting in deeper lying vessels. Furthermore, these patients often have curved arteries and calcified atherosclerotic plaques, making ultrasound imaging in general much more challenging. Finally, these patients often have irregular heartbeats or monophasic flow waveforms, with stagnant blood flow during diastole. While the mentioned anatomical challenges were expected beforehand, the escalated microbubble destruction due to stagnant flow in some patients came as a surprise.

Another challenge for imaging patients with vascular diseases is the presence of stents. In both CT and MRI scanning, stents can lead to imaging artefacts. Fortunately, blood flow quantification inside stents was shown to be feasible using echoPIV, without substantial problems (see chapter 7).

After feasibility testing in healthy volunteers and patients, it’s time to prove the added benefit of the technique that is to be implemented into clinical practice. In chapter 6, we made a good start by showing that flow disturbances can be detected with echoPIV-derived flow parameters, in regions that are not identified as problematic with conventional duplex US. However, more studies are needed in the future to expand and improve upon these findings.

**Future perspectives**

Even though some clinical benefits were already shown in this thesis, a potentially much more groundbreaking application of echoPIV (and other flow quantification techniques) is the prediction of atherosclerotic disease progression and stent patency (or other forms of clinical outcome).

To investigate whether this is feasible, a 2-year clinical follow-up will be performed on all 97 patients that received echoPIV measurements in this thesis. Duplex ultrasound will be used to monitor the severity of the lesion in unstented patients and stent patency will be monitored in stented patients. Medical files will be periodically screened to register any surgical or endovascular (re-)interventions and adverse events.

After 2 years, this data will be used to investigate the relation between the echoPIV-derived flow patterns at baseline and the clinical outcome of the patients. This could yield valuable new insights into the prognostic value of echoPIV. Again, additional studies in larger cohorts will be needed to strengthen these findings.
If the added benefit of an imaging technique in a clinical setting is proven, the final challenge is a successful implementation into clinical practice, which is not self-evident. In the case of echoPIV-derived flow patterns, cut-off values have to be found that lead to the best prediction of clinical outcome. Furthermore, several improvements were suggested in chapter 6 that would ease the implementation of echoPIV into clinical practice. This includes the development of new contrast agents with improved longevity and resonant properties that are better matched to the US frequency if specific imaging sequences. Together with new US acquisition sequences this may lead to enhanced contrast visibility on the HFR-CEUS images, even in patients with stagnant blood flow where the images currently suffer from microbubble destruction. Finally, three-dimensional matrix probes with true real-time flow quantification would allow visualization of the full flow field in complex three-dimensional vessel geometries, with direct feedback to optimize imaging location and acquisition settings.

These mentioned hardware or processing improvements must not only be developed in the lab. Before they can be used in daily clinical practice, they also have to be implemented into the next generation of standard clinical ultrasound devices. In the case of new contrast agents, these have to be approved by regulatory authorities, which could take a decade, or more. Here lies an important role for companies in the medical devices industry, that at some point have to turn fundamental or translational science, funded by governments and charitable organizations, into profitable products. However, to achieve the best results in terms of high-quality cost-effective patient care, close co-operation is required between these companies, hospitals and scientific institutions.

Even if a new imaging technique is finally available on commercial devices with the appropriate medical certification, it still needs to be incorporated into clinical guidelines before the practice will be adopted on a large scale. A striking example here is the use of contrast-enhanced ultrasound (CEUS) for the detection of endoleaks after endovascular aneurysm repair, which was introduced in Rijnstate during my PhD. Even though 1) the contrast agents have been used for decades, 2) most current-generation clinical US machines include contrast imaging sequences and 3) many studies have shown the benefits of CEUS compared to CT imaging, this technique is not yet widely used in Dutch hospitals. This could be due to the fact that none of the international vascular guidelines recommend the technique, possibly due to its operator dependency in combination with inexperienced hospital staff.

All these hurdles on the road from the lab to the clinic may seem quite daunting and sometimes, they are. However, many reasons exist to be hopeful for the future of echoPIV. Assuming the technique will be developed further, its added clinical value becomes clear and it is successfully implemented into daily clinical practice (i.e. if everything goes according to plan and all the mentioned hurdles will be overcome), this technique could drastically improve the treatment of patients with AIOD. Its greatest strength will lie in personalized treatment and follow-up, of which several examples are given here.
First, if the future growth rate of a lesion can be predicted based on the blood flow, this can be used to help decide when endovascular treatment, with or without a stent, should be performed. This could be before symptoms are severe enough for intervention according to the current guidelines, but when they are predicted to worsen on short-term. In this way, complex procedures on lesions that are difficult to pass, that typically lead to lower success rate, can be prevented. Alternatively, lesion severity could be estimated more accurately based on the pressure gradient derived from the flow field, eventually assisted by advanced CFD algorithms and artificial intelligence. This could lead to a better informed choice on which of multiple lesions to treat first, instead of the current standard protocol of opting for the most proximal one.

During (endo)vascular surgery, real-time flow quantification with ultrasound could be used to evaluate the placement of the stent or bypass graft. Flow disturbances could then be immediately addressed, improving the outcome of the procedure.

And finally, during follow-up after treatment, flow quantification could be used to adjust the frequency of follow-up visits, with patients showing an undisturbed flow pattern having limited follow-up. On the other hand, patients that are presented with flow disturbances will be offered more regular visits, to safeguard the patency of the stents and facilitate early re-intervention in cases where this is necessary.

**General conclusions**

The work presented in this thesis shows that current diagnostic techniques for patients with AIOD are unable to quantify local hemodynamic parameters, that are crucial for the full evaluation of the disease.

One of the techniques that can be used to quantify these blood flow parameters is echoPIV. In this thesis, multiple studies were performed to test the feasibility and clinical application of echoPIV, to bring this technique from the bench to the patient’s bedside. In healthy volunteers, the acquisition scheme and processing was optimized for in vivo use and validated with 4D flow MRI. In patients with AIOD, EchoPIV was shown to be feasible, even inside a variety of stents placed in the aortoiliac region. Furthermore, it was shown that echoPIV can be used to quantify blood flow disturbances in regions that would not normally be identified as problematic with duplex US.

If the echoPIV technique is further developed and successfully implemented into clinical practice, this could lead to a paradigm shift in the treatment of AIOD, where local flow parameters guide the monitoring and treatment of the disease.
Chapter 9
Dutch summary
Bloedstroming analyseren in aortoiliacale slagaderen: van het lab naar de patiënt

Aortoiliacaal stenotisch (vernauwend) vaatlijden (AISV), is een vorm van perifeer vaatlijden in de distale aorta en iliacale slagaderen. De prevalentie van AISV neemt toe met de leeftijd, hoewel het grootste deel van de patiënten geen symptomen ervaart. De meest voorkomende klacht bij AISV zijn etalagebenen, oftewel pijn in de benen na een korte tijd lopen, die weer afzakt bij kort uitrusten. Uiteindelijk kan de ziekte leiden tot kritieke ischemie van de ledematen en in sommige gevallen tot gedeeltelijke of volledige amputatie van een been.

De ziekte kan worden behandeld middels gesuperviseerde looptraining, maar als dit geen effect heeft, of als de klachten sterk invaliderend zijn, is chirurgische of endovasculaire behandeling geïndiceerd. Bij endovasculaire behandelingen worden vaak een of meerdere stents geplaatst. Bij uitgebreide laesies rondom de aortabifurcatie is meestal een configuratie van meerdere stents vereist, zoals de “Covered Endovascular Reconstruction of the Aortic Bifurcation” (CERAB).

Hoewel de pathologische mechanismen achter AISV sterk worden beïnvloed door lokale bloedstroming, speelt dit geen belangrijke factor bij de huidige diagnose en behandeling van de ziekte. Nauwkeurige quantificering van deze bloedstroompatronen zou daarom de behandeling van patiënten met AISV kunnen verbeteren. Een veelbelovende techniek hiervoor is echoPIV, dat bestaat uit een combinatie van high-frame-rate contrast-enhanced ultrasound (HFR-CEUS) en particle image velocimetry (PIV). Het belangrijkste doel van dit proefschrift was om de haalbaarheid en klinische toepassing van echoPIV te onderzoeken, om deze techniek vanuit het lab naar de patiënt te brengen.

In hoofdstuk 2 werd de invloed van lokale bloedstroompatronen op AISV in detail beschreven. Deze invloed is belangrijk bij de ontwikkeling en progressie van atherosclerose en zou daarom een belangrijke rol kunnen spelen bij de monitoring van de ziekte. Bovendien beïnvloedt de lokale bloedstroom de doorgankelijkheid van stents. Deze kennis kan mogelijk gebruikt worden om endovasculaire behandelingen en postoperatieve zorg te verbeteren.

Huidige diagnostische technieken, zoals conventionele duplex-echografie en CT-angiografie, werden tevens besproken in hoofdstuk 2, inclusief hun beperkingen bij de evaluatie van de lokale bloedstroom. Hierbij werd duidelijk dat er behoefte is aan verbeterde methoden om de ernst van de laesie in te schatten of om ziekteprogressie en stent doorgankelijkheid te voorspellen, op basis van de lokale bloedstroom. Dit hoofdstuk geeft een uitgebreid overzicht van nieuwe technieken voor de kwantificatie van bloedstroom die voor deze toepassingen kunnen worden gebruikt, waaronder op ultrageluid gebaseerde methoden zoals echoPIV, 4D-flow MRI en computational fluid dynamics.
Deze nieuwe technieken, die kwantificering van de bloedstroom in vivo mogelijk maken, zijn nog niet algemeen beschikbaar in de dagelijkse klinische praktijk en kunnen daarom niet worden gebruikt om patiënt-specifieke beslissingen omtrent de behandeling te ondersteunen. Echter kan nog steeds waardevolle algemene kennis worden vergaard door gebruik van in vitro modellen.

In hoofdstuk 3 werden lokale bloedstroompatronen gekwantificeerd met laserPIV, in twee vereenvoudigde modellen van de aortabifurcatie, met en zonder een CERAB-configuratie. Het doel van deze studie was het onderzoeken van een veelvoorkomend klinisch probleem: Het opnieuw afsluiten van een stent als gevolg van verslechterde distale uitstroom.

Deze studie toonde aan dat de lokale wandschuifspanning meer afneemt in de CERAB-configuratie, in vergelijking met het controlemodel, wanneer een distale stenose wordt toegevoegd aan het model. Deze bevindingen zouden de relatie tussen distale uitstroom en re-occlusie van de stent kunnen verklaren, wat waardevolle kennis is bij de ontwikkeling van nieuwe endovasculaire apparaten of behandelprotocollen.

De stip aan de horizon is echter een nauwkeurige kwantificering van de bloedstroom bij patiënten. Om nieuwe flow-kwantificatie technieken in het ziekenhuis te introduceren, moet de haalbaarheid van deze technieken worden geëvalueerd en moet hun toegevoegde waarde worden aangetoond. In hoofdstuk 4 en 5 wordt de haalbaarheid van echoPIV beschreven bij gezonde vrijwilligers. De haalbaarheid en klinische toepassing van echoPIV bij patiënten met aorto-iliacale aandoeningen, met en zonder stents, wordt onderzocht in hoofdstuk 6 en 7.

**Hoofdstuk 4** beschrijft een parameter studie naar verschillende instellingen voor data acquisitie en analyse. Deze studie resulteerde in verschillende bevindingen die vervolgens werden gebruikt om de echoPIV-methode verder te optimaliseren voor gebruik in patiënten (hoofdstuk 6 en 7). Allereerst vond er vernietiging van microbellen plaats in alle metingen, behalve diegene met de laagste ultrageluid intensiteiten, wat betekent dat er een delicaat evenwicht is tussen het verlies van het contrast materiaal en een adequate signaal-ruisverhouding.

Bovendien bleek op Singular Value Decomposition (SVD) gebaseerde clutter-filtering effectiever in het onderdrukken van weefseksignaal, vergeleken met amplitude modulatie, een geavanceerd acquisitieschema dat niet-lineaire harmonische signalen van de microbellen kan versterken t.o.v. lineaire signalen. Met andere woorden, analyse van de spatio-temporele kenmerken van de microbellen is momenteel effectiever dan de detectie van hun harmonische frequentie-inhoud. Toekomstige ontwikkelingen die dit in de toekomst zouden kunnen veranderen, worden besproken in hoofdstuk 6.
In Hoofdstuk 5 werden echoPIV-afgeleide stroomsnelheden van 8 vrijwilligers vergeleken met een referentie, 4D flow MRI. Over het algemeen zijn de gemeten pieksnelheden tijdens de systole en het gehele snelheidsprofiel door de tijd zeer vergelijkbaar in beide technieken, wat de validiteit van echoPIV aantoonde. Bij 1 vrijwilliger die een stilstand van de bloedstroom vertoonde tijdens de diastole, vertoonden beide technieken een kleine en kortstondige recirculatie op exact hetzelfde moment (einde van de systole) en dezelfde locatie (oorsprong van de linker arteria iliaca). Dit gaf vertrouwen in het vermogen van echoPIV om deze gedetailleerde stroompatronen te kwantificeren. Bij 2 vrijwilligers vertoonden echoPIV een achterwaartse stroming tijdens diastole, terwijl 4D-flow MRI dat niet deed. Hoewel deze metingen op verschillende momenten zijn gedaan, denken we dat dit te wijten is aan het middelen van meerdere hartslagen in 4D-flow-MRI en dat juist echoPIV in deze gevallen de ware snelheden laat zien.

In Hoofdstuk 6 werd de haalbaarheid en klinische toepassing van echoPIV bij patiënten met aorto-iliacale aandoeningen onderzocht. Deze studie toonde aan dat kwantificering van de bloedstroming met echoPIV bij bijna alle patiënten haalbaar is, hoewel er bij meer dan de helft van de patiënten enkele beperkende problemen optreden die de volledige visualisatie (tijdens de volledige hartcyclus of in het gehele afgebeelde vaatsegment) tot op zekere hoogte beperken. Bovendien kunnen van echoPIV afgeleide parameters (vectorcomplexiteit en vorticiteit) worden gebruikt voor het detecteren van verstoringen in de bloedstroming, in regio’s die niet worden bestempeld als problematisch bij gebruik van conventionele duplex echografie.

In hoofdstuk 7 werd de robuustheid van echoPIV onderzocht, door de haalbaarheid te testen van bloedstroom kwantificering in stents die in het aortoiliacale gebied zijn geplaatst. Deze studie toonde aan dat echoPIV haalbaar is in verschillende veelgebruikte stents. Bovendien veroorzaken deze stents geen afname in beeldkwaliteit of prestaties van het echoPIV-algoritme. Deze bevindingen tonen de potentiële meerwaarde van echoPIV in de klinische workflow, wat de resultaten van endovasculaire behandeling in het aorto-iliacale kanaal zou kunnen verbeteren.

Concluderend, bleek EchoPIV dus toepasbaar in patiënten met AISV, zelfs in stents die in het aortoiliacale gebied worden geplaatst. echoPIV kan tevens worden gebruikt om bloedstroomverstoringen te kwantificeren in regio’s die normaal niet als problematisch zouden worden bestempeld met de huidige diagnostiek. Als de echoPIV techniek verder wordt ontwikkeld en met succes wordt geïmplementeerd in de klinische praktijk, kan dit leiden tot een verbeterde behandeling van AIOD, waarbij lokale flowparameters de monitoring en behandeling van de ziekte bijsturen.
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AA</td>
<td>Abdominal aneurysm</td>
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<tr>
<td>AIOD</td>
<td>Aortoiliac occlusive disease</td>
</tr>
<tr>
<td>AM</td>
<td>Amplitude modulation</td>
</tr>
<tr>
<td>CBR</td>
<td>Contrast-to-background ratio</td>
</tr>
<tr>
<td>CEUS</td>
<td>Contrast-enhanced ultrasound</td>
</tr>
<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
</tr>
<tr>
<td>CTA</td>
<td>Computed tomography angiography</td>
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<td>DR</td>
<td>Disruption ratio</td>
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<tr>
<td>DUS</td>
<td>Duplex ultrasound</td>
</tr>
<tr>
<td>HFR</td>
<td>High-frame-rate</td>
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<tr>
<td>MI</td>
<td>Mechanical index</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>OSI</td>
<td>Oscillatory shear index</td>
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<tr>
<td>PAD</td>
<td>Peripheral arterial disease</td>
</tr>
<tr>
<td>PC-MRI</td>
<td>Phase contrast magnetic resonance imaging</td>
</tr>
<tr>
<td>PI</td>
<td>Pulse inversion</td>
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<tr>
<td>PIV</td>
<td>Particle image velocimetry</td>
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<tr>
<td>SVD</td>
<td>Singular value decomposition</td>
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<tr>
<td>TAWSS</td>
<td>Time averaged wall shear stress</td>
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<tr>
<td>TO</td>
<td>Transverse oscillations</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound contrast agent</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VFM</td>
<td>Vector flow mapping</td>
</tr>
<tr>
<td>WSS</td>
<td>Wall shear stress</td>
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</tbody>
</table>
References


[63] S. F. C. Stewart, “Effects of transducer, velocity, Doppler angle, and instrument


Deze thesis had nooit tot stand kunnen komen zonder de hulp van alle mensen om me heen. In de hoop niemand te vergeten, wil ik bij deze een poging doen om mijn waardering uit te spreken aan iedereen die een bijdrage heeft geleverd aan mijn promotieonderzoek.

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Biography

Stefan Engelhard was born on the 25th of July in Oirschot. In 2008 he graduated from the Heerbeeck College in Best. After spending a winter in Austria as a snowboard instructor, he started studying Technical Medicine at the University of Twente. During this period he also joined the Dutch National reserve corps. Stefan did his graduation internship at the Rijnstate hospital in Arnhem, working on flow quantification in healthy volunteers. After his graduation, he started his PhD as a continuation of the research project, primarily working on patient studies. In November 2021, Stefan started a new job as an endovascular product specialist at JOTEC GmbH / Cryolife, Inc. This was a small step from occlusive to aneurysmatic vascular disease and a giant leap from the academic to the corporate world…