Doctoral theses at NTNU, 2022:81

Erik Mulder Pettersen

Earlybird

A new approach to vascular assessment

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

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Trondheim, April 2022

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earlybird

SØRLANDET SYKEHUS

NTNU

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All truth passes through three stages.

First, it is ridiculed.

Second, it is violently opposed.

Third, it is accepted as self-evident.

Misattributed statement [1, 2] of

Arthur Schopenhauer,

German philosopher.

Contents

Norsk sammendrag

Formålet med doktorgradsarbeidet var å utforske mulige kliniske bruksområder innenfor karhelse for et nyutviklet, høy-sensitivt, ultralyd Doppler system (earlybird). Enheten er utviklet ved Institutt for sirkulasjon og bildediagnostikk, NTNU, ved professor Hans Torp. Earlybird består av ulike ultralydprober med et relativt stort areal, en skanner og et tilpasset brukergrensesnitt. Egenskapene gjør den enkel å plassere over karstrukturer av interesse. Earlybird-prosjektet er del av en større innovasjon- og kommersialiserings-prosess av den underliggende ultralyd-teknologien, som omfatter andre kliniske bruksområder.

I studie I ble earlybird sammenlignet med laser og pulset Doppler. Friske personer ble utsatt for ulike fysiologiske tester som igangsetter en vasomotorisk respons. Det ble funnet en god overenstemmelse mellom de ulike målemetodene (artikkel I). Earlybird virker til å kunne fange opp endringer i perifer blodgjennomstrømning hos friske personer.

I studie II, inkluderte man en behandlingsmetode som tar i bruk intermitterende undertrykk (FlowOx™) for å behandle pasienter med redusert blodgjennomstrømning til beina. En del av pasientene med anstrengelsesutløste gangsmerter er ikke i stand til å delta i anbefalte treningsprogram, og det er derfor interessant å vurdere ikke-invasive behandlingsmetoder. I denne randomiserte studien var 63 pasienter tilgjengelige for vurdering etter endt oppfølgingstid. Det ble funnet at pasienter som ble behandlet i 12 uker med 40 mmHg intermitterende undertrykk hadde 50 meter gjennomsnittlig økning i smertefri gangdistanse, sammenlignet med de som ble behandlet med 10 mmHg (artikkel II). For å vurdere sekundære utfall av studien (artikkel III) ble målinger av blodstrømshastigheter, målt med earlybird, gjennomført før og etter den 12-ukers lange behandlingsperioden. Man fikk gode og pålitelige målinger av blodgjennomstrømningen. Dette viser at earlybird også kan måle endring i blodsirkulasjon hos pasienter med perifer arteriell sykdom. Målingen ble brukt til å

analysere effekter på blodstrømmen, forårsaket av behandling med intermitterende undertrykk. Det ble bekreftet at intermitterende undertrykksbehandling gir en umiddelbar økning i blodstrømshastigheten. Det kan også virke som om den positive effekten sett ved behandling med FlowOx™, delvis kan forklares av økt aktivitet av glattmuskulatur i karveggen tilsvarende arteriolene.

Blodstrømshastigheter ble overvåket i sanntid med earlybird i det aktuelle beinet som gjennomgikk endovaskulær behandling (studie III, kongress abstrakt I). Først etter ballong-dilatasjon av en stent, plassert i en bekkenokklusjon, får man en økning i blodstrøm. Earlybird kan være et nyttig verktøy for peroperativ klinisk beslutningsstøtte. Imidlertid er det nødvendig med flere studier for å vurdere hvilke hemodynamiske parametere og terskelverdier som kan forutsi kliniske endepunkter.

I studie IV har man vist at earlybird kan være et fremtidig verktøy til å måle blodstrøm i arteriovenøse fistler til bruk for hemodialyse (artikkel IV). Selv om det er vist at overvåkning av arteriovenøse fistler kan redusere tromboseringsraten, diskuteres nytteverdien av slike overvåkningsregimer. Det er foreslått at analyse av blodstrøms-trender kan være nyttige. Den største begrensningen til earlybird vil være at den ikke tillater vinkelkorrigering av blodstrømshastigheten. Videreutvikling av earlybird, spesielt med tanke på å gjøre den vinkeluavhengig, vil kunne øke brukervennligheten og forbedre nøyaktigheten.

Earlybird har vist seg å kunne måle og overvåke perifer blodsirkulasjon. Earlybird kan være et potensielt fremtidig verktøy for klinisk beslutningsstøtte under endovaskulære behandling og for overvåkning av arteriovenøse fistler brukt som tilgang til hemodialyse. Ytterligere utvikling av den underliggende teknologien og programvare vil kunne øke brukervennligheten og medføre tilpasning av earlybird til et bredt spekter av kliniske bruksområder.

Preface and acknowledgement

Earlybird is part of an innovation and commercialization project based on a novel ultrasound Doppler device. The underlying technology is developed at Department of Circulation and Medical Imaging at NTNU, by Professor Hans Torp. Although this is a technological academic environment, it is structurally located between floors occupied by Trondheim university hospital (St.Olavs), which is filled with relevant patient categories and health care personnel. This lay the ground for valuable interaction between clinicians, technology-based scientist, and developers. Based on the technological innovation, a search for clinical applications was initiated, expanding its involvement to market surveyors, product engineers, patent lawyers and animators. In the same manner, a valuable interaction between the Section of Vascular Investigations at Oslo university hospital and Otivio has been developed to further examine intermittent negative pressure therapy as delivered by FlowOx™.

I am grateful for the introduction, and inclusion, to these stimulating environments, made possible by my main supervisor and friend, Arne Seternes. He has facilitated the progress of this thesis with firm support, stimulating both mind and palate. I want to thank the inspirational involvement of supervisor Professor Hans Torp, always eager to answer “simple” questions by a non-technologist and providing “automagic” solutions. The positive and motivational attitude of supervisor and Professor Jonny Hisdal, who always has time to share his insight to the mysteries of vascular physiology, was crucial during the “darkest times” of this thesis. The differences in professional background of my supervisors have actively supported the work in this thesis.

The contributions of all co-authors are greatly appreciated. Especially I enjoyed the collaboration with Henrik Hoel. The positive easy-going attitude combined with an academic mindset was the key to being able to carry out recruitment and follow-up, including home visits, of the RCT on intermittent negative pressure. Thanks to Eivind Andersen at the Technical Transfer Office AS, NTNU, for guidance through the application process for financial aid to support this thesis.

I would like to thank the dialysis unit at Sørlandet Hospital Kristiansand, especially the efforts of Anne Margrethe Myhrmoen and Marianne Klausen, for their facilitation in the study on arteriovenous fistulas. The inclusion and testing of patients from Sørlandet Hospital in the randomized controlled trial wouldn't have been possible without the support of the Department of Physiotherapy and the invaluable effort of physiotherapist Lina Krohg.

This thesis would not have been possible without the support and adaptability of my colleagues at the Section of Vascular Surgery, at Sørlandet Hospital. I am especially grateful for the facilitation by Andreas Nygaard in the final phase of the project. I also must thank the Department of Surgery and Sørlandet Hospital Kristiansand, personalized by the head of department, Paula Axelsen. I have valued the lunches and collegial community with the vascular surgeons at St.Olavs Hospital. Broadening of my professional network has been motivational.

Thanks for the always warm and familiarly reception at the Seterneses during my stays in Trondheim.

Thanks to my children, Kasper, Tuva, and Solveig, for being bright spots during everyday life and giving me diversion during tense periods of the thesis. I am grateful for the efforts and support of my loving wife, Hedda, whom with care and love is the "glue" in our family.

Thanks to my parents, Renny and Harald. Thanks to all my friends for putting up with my absence of mind and their patience through this all-consuming project of mine.

The work has been carried out at the Department of Vascular Surgery, St Olavs Hospital, Department of Circulation and Medical Imaging, NTNU, Section of Vascular Investigations, Oslo university hospital, and Sørlandet Hospital Kristiansand between 2017 and 2021. A grant was provided by NTNU Innovation, at the Faculty of Medicine and Health Sciences.

Kristiansand, December 2021

List of publications

Papers

I Pettersen EM, Avdal J, Hisdal J, Torp H, Seternes A. Validation of a novel ultrasound Doppler monitoring device (earlybird) for detection of microvascular circulatory changes. Clin Hemorheol Microcirc. 2020;74(4):429-440. doi: 10.3233/CH-190707. PMID: 31743988. Reprinted with permission, IOS Press ©.

II Hoel H, Pettersen EM, Høiseth LØ, Mathiesen I, Seternes A, Hisdal J. A randomized controlled trial of treatment with intermittent negative pressure for intermittent claudication. J Vasc Surg. 2021 May;73(5):1750-1758.e1. doi: 10.1016/j.jvs.2020.10.024. PMID: 33899743. Reprinted with permission Elsevier ©.

III Pettersen EM, Hoel H, Hisdal J, Torp H, Seternes A. The effect of 12-week treatment with intermittent negative pressure on blood flow velocity and flowmotion, measured with a novel Doppler device (earlybird). Data from a randomized controlled trial in patients with peripheral arterial disease. Submitted.

IV Pettersen EM, Avdal J, Fiorentini S, Salvesen Ø, Hisdal J, Torp H, Seternes A. Validation of a novel ultrasound Doppler monitoring device (earlybird) for measurements of volume flow rate in arteriovenous fistulas for hemodialysis. The Journal of Vascular Access. December 2021. doi:10.1177/11297298211060960 Reprinted with permission, SAGE Publishing ©.

Congress abstract

I Pettersen, E. M., Avdal, J., Hisdal, J., Torp, H., & Seternes, A. (2020). Earlybird - A Novel Ultrasound Doppler Monitoring Device - Potential Future Application in Per-Operative Monitoring. EJVES Vascular Forum, 48, 40–41. https://doi.org/10.1016/j.ejvsvf.2020.07.006

List of acronyms and abbreviations

ABI	ankle-brachial index
AVF	arteriovenous fistula
CW Doppler	continuous wave Doppler
CLTI	critical limb threatening ischemia
DUS	duplex ultrasound
ESVS	European Society for Vascular Surgery
FMD	flow mediated dilation
INP	intermittent negative pressure
IPC	intermittent pneumatic compression
ICC	intraclass correlation coefficient
LDF	laser Doppler flowmetry
LEAD	lower extremity arterial disease
MWD	maximum walking distance
NTNU	Norwegian University of Science and Technology
PWD	pain-free walking distance
PAD	peripheral arterial disease
PW Doppler	pulsed wave Doppler
SET	supervised exercise treatment
NIRS	transcutaneous near-infrared spectroscopy
$TcPO_2$	transcutaneous oxygen pressure
V_{mean}	mean blood flow Doppler velocity
VFR	volume flow rate
ZNCC	zero-mean normalized cross-correlation

Summary

The aim of the thesis was to explore and identify possible clinical applications within vascular health for a novel, high-sensitivity, ultrasound Doppler system (earlybird). The unit was developed by Professor Hans Torp, at the Department of Circulation and Imaging, NTNU. Earlybird consists of an ultrasound probe with a relatively large area, scanner, and a customized user interface. The device is designed to monitor peripheral blood flow simultaneously and continuous in a depth down to 40 mm. It allows easy placement over vessel structures of interest. The Earlybird project is part of a larger innovation and commercialization process of the underlying ultrasound technology, which includes other clinical applications.

In a proof-of-concept study (study I, paper I), it was demonstrated that earlybird correlates well with laser Doppler flowmetry and pulsed-wave Doppler to assess microcirculatory function in healthy subjects [3]. This study validates earlybird's ability to assess peripheral blood circulation.

The second study (study II, paper II and III) includes an innovational treatment-device (FlowOx™) for patients with peripheral arterial disease. A proportion of patients with intermittent claudication, are not able to participate in exercise programs due to lack of motivation or comorbidity. FlowOx™ incorporates intermittent negative pressure (INP) to increase peripheral blood circulation. In a randomized sham-controlled trial, patients with intermittent claudication were treated for 12 weeks with either 40 mmHg or 10 mmHg INP. A mean treatment effect of increase in pain-free walking distance of 50 meters was found, in favor of the 40 mmHg INP treatment group (paper II) [4]. Earlybird-recordings were used to explore secondary outcomes (paper III). An immediate increase in blood flow velocities was observed during the INP treatment. Analyses of flowmotion characteristics of endothelial, sympathetic, and myogenic activity, showed a difference in change of myogenic activity between the groups after 12 weeks of treatment with INP. This finding suggests an involvement of vascular

smooth muscle cells of the arterioles, and it may contribute to the understanding of the mechanism of action of INP. The ability of earlybird to monitor and assess blood flow velocities, was confirmed in patients with peripheral arterial disease.

In a pilot study (study III, congress abstract I) blood flow velocities were monitored during an endovascular procedure. Earlybird detects changes in blood flow velocities in real-time. Earlybird could be a valuable tool for periprocedural decision making, to guide the clinician in what extent to revascularize a limb [5]. Further studies are needed to determine hemodynamic properties that can be associated with clinical endpoints.

Although controversial, the surveillance of volume flow rate (VFR) in arteriovenous fistulas (AVF) for hemodialysis could increase their patency. Earlybird's ability to be a potential future tool for surveillance of VFR in AVFs was evaluated in an experimental and clinical setting (study IV, paper IV). VFR-measurements were automatically derived from earlybird's flow velocity recordings, and was compared to duplex ultrasound, and calibrated VFR. Earlybird was found to be a feasible tool for evaluating VFR in AVFs, with a strong correlation and good agreement between the methods used [6]. Further development, especially to overcome the limitations with angle dependency, may increase user-friendliness and further improve the accuracy. Earlybird could be a potential valuable tool for surveillance of VFR.

FlowOx™ is a promising device, supplementary to standard care of peripheral arterial disease. Earlybird has been demonstrated to be a feasible device to assess blood flow in healthy individuals, as well as in patients with a range of clinical challenges within the broad segment of vascular disease. Earlybird could be a future tool for clinical decision making during endovascular treatment and a future promising tool for surveillance of hemodialytic vascular access. Further technical and software development of earlybird, may increase user-friendliness and allow for a wide range of clinical applications.

Introduction

Innovation in healthcare

Innovation can be defined as the development of new ideas, design, or products [7]. A more comprehensive definition of innovation is provided by West (1989): "the intentional introduction and application within a role, group, or organization, of ideas, processes, products or procedures, new to the relevant unit of adoption, designed to significantly benefit the individual, the group, or wider society" [8]. The definition is often used to define innovation in healthcare [9, 10], and is adapted from business, technical, and marketing industries [11]. An innovation must be significantly different and provide substantial benefit [11]. The World Health Organization Innovation Group (WHIG) defines health innovation as [12]:

- Health innovation is to identify new or improved health policies, systems, products and technologies, and services and delivery methods that improve people's health and wellbeing.
- Health innovation responds to unmet public health needs by employing new ways of thinking and working with a special focus on the needs of vulnerable populations.
- Health innovation aims to add value in the form of improved efficiency, effectiveness, quality, sustainability, safety and/or affordability.
- Health innovation can be preventive, promotive, palliative, curative, rehabilitative and/or assistive care.

Modern society depends on implementing innovations to overcome the increasing financial burden of healthcare costs [9, 11, 13]. Governments have great expectations toward the potential of the use of medical devices, sensor technology, digital population services, home treatment programs, as well as systems for interaction and cooperation between the citizen and different healthcare providers [9, 14]. The use of emerging health technology to achieve a sustainable development of healthcare is

proposed through; digitalizing health; do-it-yourself diagnostics; synthetic biology; biomaterials; genome sequencing; bioprinting; robotics and artificial intelligence; sensors and wearable devices and big data [15].

Innovation process

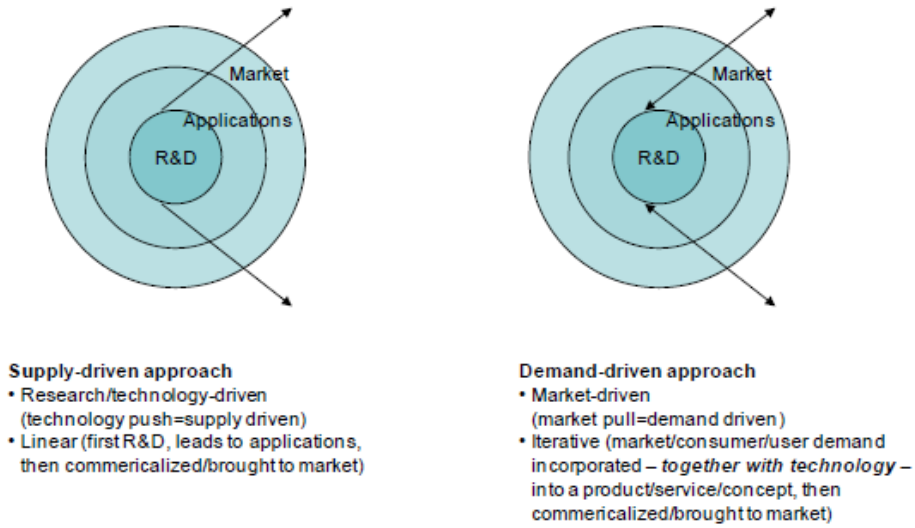

Figure 1: Supply and demand-driven innovation [16].

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Drivers of innovation has been classified as price, research or technology and user, consumer, or market [17]. In technical health associated development environments, a focus has primarily been on a "supply-driven approach"[16] , also defined as technology-driven innovation [18, 19], figure 1. The innovation classically starts in the research and development department. A large investment in research and development does not necessarily lead to a high innovation performance [20]. It was necessary to acknowledge the end-users need, leaving the traditional innovation drivers of price and quality, to increase competitiveness in an increasing global market [16]. With the goal of implementing and collecting the benefit of the innovation, user-driven innovation process could speed up the process. User-driven innovation incorporates: 1) "the use of different methods to understand not only stated but also latent consumer needs";

and 2) “a different “structure of investments” and more strategic focus on understanding and developing solutions to meet consumer needs” [20]. Solutions to a defined problem are conceptualized and tested before implementation, as described in the “innovation wheel”[18], figure 2. Historically, healthcare innovations have a tradition for involving the end-user in its process, at least to a larger degree than in market, industry or business related innovation process [19].

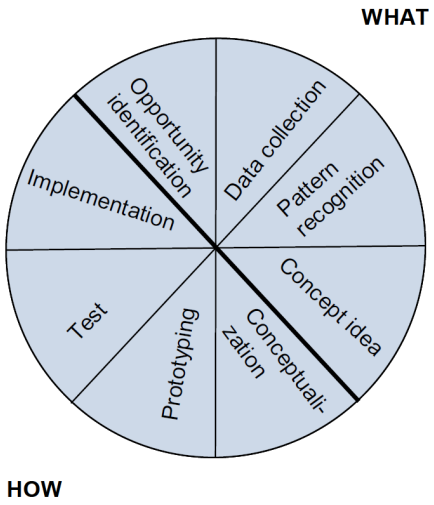

Figure 2: Innovation wheel [18].

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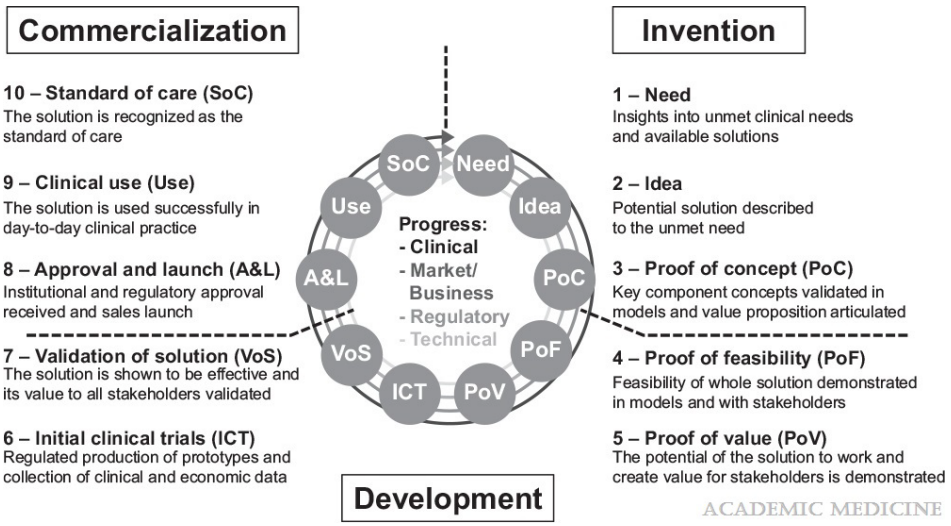
The innovation process could be defined in three stages [10]. A service or product could be designed after identifying the customers’ wants or needs (stage I). The product is tested, adapted, and improved to meet the market demands (stage II). To proceed to the next stage (stage III), “the possible service or product” must be created. The customer is not aware of the need, and rather to ask, “what do the customer want?” the innovator must ask “what would they love?” [10]. A new market may be created.

Translating academic advances into clinical practice and outcome can be difficult, risky, expensive, and is often poorly understood by the involved parts [21, 22]. To decrease risk and improve efficiency of the innovation process, the Consortia for Improving

Medicine with Innovation & Technology (CIMIT) developed the Healthcare Innovation Cycle [22, 23], figure 3. It is a framework to guide teams through the development process, from innovation of a solution for a clinical unmet need, to becoming the standard of care. The circle should be seen as a spiral, and for every completed cycle a



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higher standard of care is reached [23]. Four key domains (Clinical, Market/Business, Regulatory and Technical) should be systematically evaluated at each milestone to reduce risk of failure [23].

Dependable on the defined area on the need for improvement, innovation can originate within the healthcare organization, in close collaboration with healthcare technology companies, or the solution can be entirely developed by an external company and then offered healthcare-providers as a service or product [10]. Innovation can be categorized in broad categories as incremental or routine, architectural, radical and disruptive [24]. Disruptive innovation add new technology, and dramatically change the market or creates new markets [10, 24]. Poor identification and understanding, delay

translation of disruptive innovations, partly by fail to recognize the barriers and overcome them to harvest the potential benefit [24].

Commercialization and diffusion of innovation

Successful implementation of healthcare innovation depends on end-users acknowledging the innovation. The adoption of innovation may theoretically follow the Bell-curve [25, 26], figure 4. First-time users are often part of the innovation collaborators [27], also described as a technology enthusiast or risk takers [26]. Further adoption of innovation is critical of the experience of the first users. Opinion leaders and innovate-seeking professionals are often early adopters. The more conservative majority are the late adopters, who starts using an innovation when it has become the standard of practice. Finally, the traditionalist or the "laggards" adopt the innovation [26].

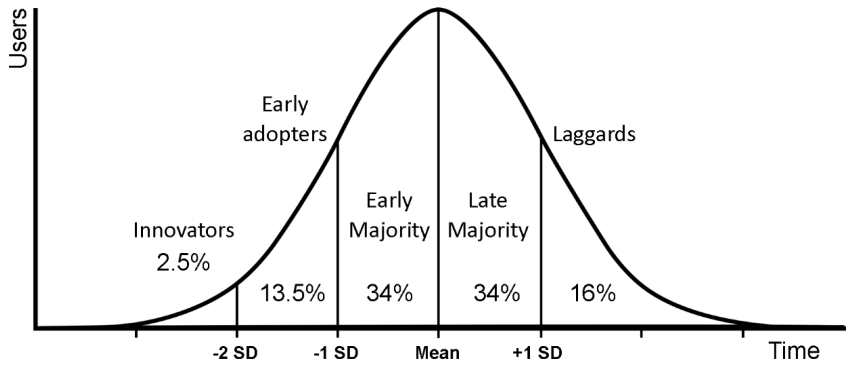


Figure 4: Diffusion of innovation, adopter categories.

Illustration: EM Pettersen, interpretation based on relevant literature [25, 26].

Diffusion of the innovation occur when the use spreads throughout the healthcare industry. Promoters, key persons with the understanding and perspective of the innovation within different segments of the innovation chain, contribute to overcome obstacles [28]. Critical factors of success for widespread use of healthcare innovations can be as described by Berwick (2003) [26]:

- Formal mechanisms to find sound innovations that should be disseminated

- Find and support innovators
- Invest in early adopters
- Make early adopter activity observable
- Trust and enable reinvention
- Create slack (including resources) for change
- Lead by example

Adoption of an innovation is almost never straightforward and is dependable on complex mechanism involving inventors, administration, funders, market needs and readiness, legislators etc. [28]. To commercialize a service or product for a broader market the intellectual property (IP) must be protected, otherwise the innovation is available for no cost [27]. On behalf of the healthcare institutions and university environments technical transfer offices are recognized as crucial to facilitate diffusion of innovative technology and by protection of patents and intellectual property (IP) rights [29, 30]. Hereby they fulfill the academic institutions' socioeconomic responsibility, the "third mission" [31]. Healthcare commercialization programs can prepare interested participants and improve the efficiency of translating healthcare innovations from clinical health care and academia into practice [32]. At the School of Health Innovation, a collaboration between the University of Oslo, Norwegian University of Science and Technology (NTNU), Karolinska Institutet in Stockholm and the University of Copenhagen, such courses/programs are established [33].

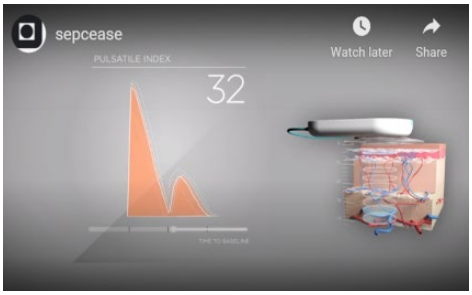
Earlybird and FlowOx™, two innovative devices.

This thesis encompasses potential areas of interest for two innovational devices within the field of vascular health: 1) a monitoring device for peripheral circulation, earlybird, and 2) a treatment device for peripheral arterial disease (PAD), FlowOx™.

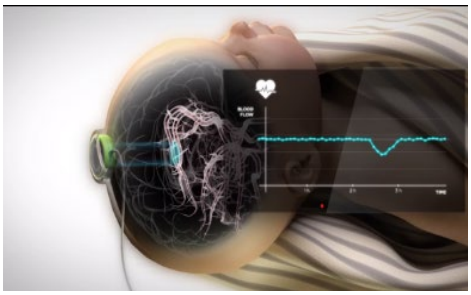
What is earlybird?

Earlybird is part of an innovation and commercialization process in search for clinical applications for a novel ultrasound Doppler device, within the broad segment of

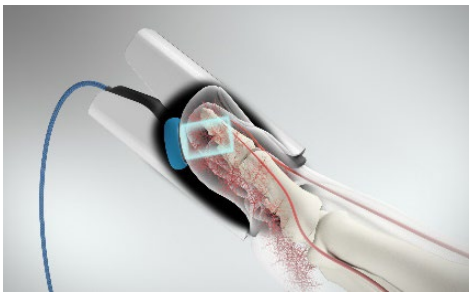
peripheral vascular disease and autonomic dysfunction. The novel ultrasound Doppler device consist of a single element highly sensitive transducer [34], scanner, and user-interface. The device was developed at the Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU) by Professor Hans Torp. Earlybird was included in a commercialization process by the Technical Transfer Office AS, NTNU. Several areas of clinical use were identified, original the following three domains of interest were explored:



SepCease – "Early warning of sepsis in the intensive care unit". Circulatory changes and dysregulation as part of a systemic inflammation response may precede sepsis [35-37]. Early detection of potential clinical deterioration may trigger adequate treatment. (Illustration used with permission, Cimon Medical©)



NeoDoppler[38, 39] – "Monitoring of cerebral circulation of neonates". Changes in cerebral function could give valuable information of the physiological state of the neonate, allowing the clinician to adjust treatment accordingly [40-42]. (Illustration used with permission, Cimon Medical©)



Earlybird – "Early diagnosis of autonomic dysfunction in diabetes". Over time, diabetic patients evolve autonomic dysfunctions affecting nerves, muscles, as well as macro and micro-circulatory regulation [43-45]. Early detection of autonomic dysfunction could initiate treatment adjustment to obtain stricter blood

glucose regulation. However, the earlybird project has evolved throughout the process, with focus on peripheral vascular disease, as stated in the following chapters of this thesis. (Illustration used with permission, Cimon Medical©).

Later in the process, new areas of clinical application have been included, VisiBeam, monitoring of fetal cerebral circulation during birth, and RescueDoppler, monitoring of carotid blood flow during resuscitation. The Technical Transfer Office, NTNU, facilitated early development of the underlying ultrasound technology and initiated the commercialization process. As a result, Cimon Medical was founded [46].

Single element highly sensitive transducer

A transducer with high sensitivity and a large aperture area of 80 mm^2, made of the piezoelectric material PZ24, was developed (Per Kristian Bolstad, University of Southeast Norway) for detection and monitoring of peripheral blood velocities [34]. Several types of transducers with different sizes, forms, and frequencies, were produced, image 1. The probe can be fixed in a case to optimize insonation angle to the underlying vessel.

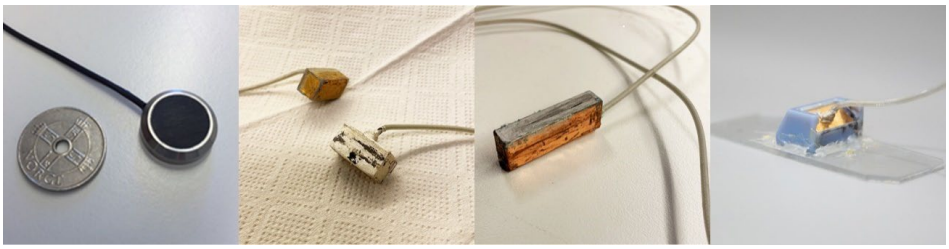


Image 1: Different transducers and an example of a mounting case.

Photo: Arne Seternes, Erik Mulder Pettersen and Karl Jørgen Marthinsen, NTNU

Scanner, user interface, and applications/tools

The transducer is connected to acquisition hardware (generic OEM Manus EIM-A produced by Aurotech Ultrasound AS, Tydal, Norway). The user interface is displayed on a computer, connected to the ultrasound scanner by an Ethernet network cable. Multi-gated Doppler signals of power Doppler M-mode and spectrograms can be

recorded for real-time visualization as well as for later post processing. In-house software was developed in MATLAB (MathWorks ® R2018a / R2020b), image 2.

Earlybird user-interface present the recorded angle-uncorrected velocity (cm/sec), pulsatile index (PI) and resistance index (RI). Different applications or tools were developed in MATLAB. Data could be exported direct to excel or other used software, defined by areas of interest according to the study performed:

- Flow curves (export to excel) – paper I
- Low, mean, and maximum Doppler velocities for predefined areas – paper I and III
- Defined INP-cycle – paper III
- M-mode and Doppler spectrograms were used to estimate vessel diameter, assuming known insonation angle an Volume Flow Rate (VFR) could be calculated – paper IV

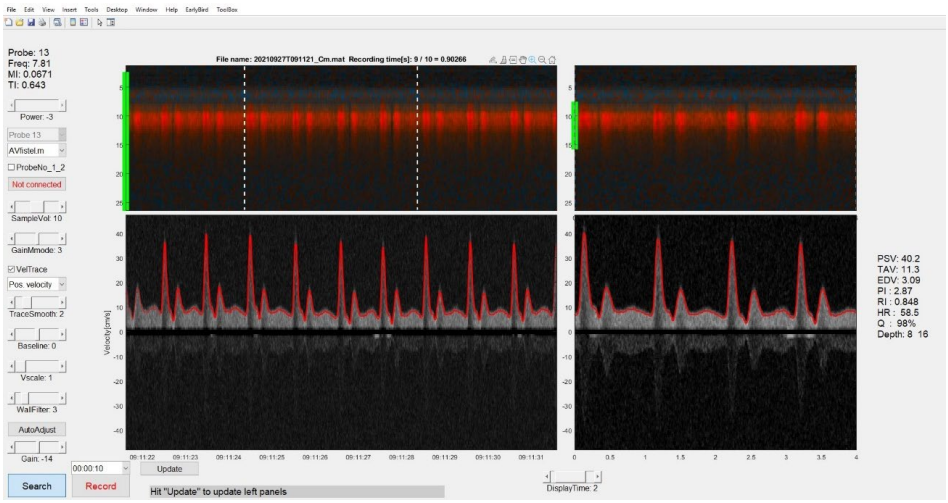


Image 2: Screenshot: earlybird user interface.

Technical development

Adaptations were made to the transducers and software to accommodate the evolving needs during the progress of the thesis. The transducers were first produced as circular elements allowing attachment to a flat surface. These probes were suitable for

monitoring blood flow moving in a close to perpendicular angle to the skin, like cerebral circulation in neonates [39]. To optimize for monitoring of blood flow velocity in vessels lying parallel to the skin, a rectangular probe was developed and mounted in a 3D-printed case for approximate 60° insonation angle. Rectangular probes with different beam-frequencies (4 - 8 MHz), were produced. Image 1 shows different transducers and an example of a mounting case.

What is FlowOx™?

FlowOx™ is a device that produce "pulsating negative pressure therapy for home use" [47]. A pressure chamber is connected to an external pump unit INP. The pump produces alternating cycles of ten seconds of 40 mmHg negative pressure and seven seconds of atmospheric pressure. This treatment protocol was found to transient increase blood flow in the treated extremity [48, 49]. The boot-design allows the distal calf, ankle, and foot to be lowered into the pressure chamber, image 3.

Image 3: Intermittent negative pressure, pressure chamber and external pump unit. FlowOx™ - Used with permission, Otivio©.

INP is used while the patient is at rest and comfortable seated. It is easily operated by the patient and is suitable for home-treatment. The device is CE-marked and used for treating disease in the peripheral micro- or microvasculature of the limbs. Otivio is a health-technology development development company, initiated to produce, commercialize, and deliver FlowOx™ to the end-user [47].

INP treatment is also described in the chapter: Non-invasive and non-pharmaceutical treatment options for PAD / *Intermittent negative pressure (INP) treatment.*

Potential areas of interest – clinical application

The main object of the thesis was to develop a new ultrasound Doppler-based device for early detection of microangiopathy in patients with diabetes mellitus and for non-invasive measurement of blood circulation during and after vascular surgery. The scope of interest has evolved through the progress of the innovation project, as result of the identified clinical applications. In this thesis, we focused on:

- Validation of earlybird as a device for monitoring peripheral arterial circulation and microcirculation.
- Clinical applications of earlybird
    - Feasibility to monitor effects on blood circulation in patients with PAD during treatment with intermittent negative pressure (INP)
    - Per-operative monitoring of endovascular revascularization
    - Surveillance of VFR in arteriovenous fistulas (AVF) in patients with hemodialysis
- The role of treatment with INP (FlowOx™) in patients with PAD.

In the following chapters, an introduction to the main clinical fields of interest and comparable devices, for both earlybird and FlowOx™ are given. There is focus on lower extremity arterial disease, non-invasive non-pharmaceutical treatment devices including INP-therapy, non-invasive monitoring devices for peripheral blood circulation

as well as clinical applications for earlybird, perioperative monitoring and surveillance of AVF.

Peripheral Vascular Disease

Peripheral vascular diseases could be defined as the medical conditions affecting all vasculature, anatomically limited to vessels outside of the heart and aorta, including arteries, veins, and lymphatic vessels [50]. Peripheral vascular diseases and PAD are often used interchangeably for atherosclerotic disease, but PAD covers a broad area of medical diseases, e.g. hypertension, endothelial dysfunction, atherosclerosis, inflammation and immunity disorders, as well as compression syndromes, aneurismal degeneration and traumatic injury [51]. In the guidelines from the European Society of Cardiology (ESC) (2017) on the diagnoses and treatment of PAD, PAD is restricted to arterial disease secondary to atherosclerotic disease [50]. In this thesis, if not mentioned otherwise, the same restriction is made for PAD and lower extremity arterial disease (LEAD).

Peripheral arterial disease (PAD) and lower extremity arterial disease (LEAD)

The prevalence of PAD is increasing and affects >235 million people globally [52, 53]. The European Region has been estimated to have the highest prevalence of PAD of 7.99% (5.10 to 13.41) [53]. There is an increasing prevalence with age [54]. LEAD is most often of atherosclerotic etiology and is associated with an increased risk of cardiovascular mortality, myocardial infarction, and stroke [50]. Patients with LEAD can be asymptomatic or have increasing burden of symptoms ranging from impaired walking capacity (intermittent claudication) to rest pain or non-healing ulcers (critical limb threatening ischemia (CLTI)) [50]. CLTI can result in gangrene, which may lead to amputation without prompt revascularization [50]. In the population-based health study in Nord-Trøndelag County, Norway (the HUNT Study) for the age group 60 to 99 years, a total prevalence of LEAD, as defined by an ankle-brachial index (ABI) < 0.9, was 7.8%. The prevalence of asymptomatic disease was 6.3%. Thus, one of five presented with

symptomatic peripheral arterial disease. These findings are in line with international prevalence-studies [55-57], while a higher prevalence was found in Sweden [58].

If the degree of reduced walking capacity, due to PAD, severely compromises daily life activity, revascularization can be considered [50]. Patients with intermittent claudication should quit smoking, optimize lifestyle risk factors and medical treatment, primarily to reduce cardiovascular mortality. First-line treatment to increase walking capacity is exercise treatment, preferably supervised exercise treatment (SET) [50]. Due to comorbidity or lack of motivation, a large proportion of patients are not suitable for exercise treatment [59-61]. Devices for non-invasive and non-pharmaceutical treatment options for PAD have therefor emerged [62].

Devices for non-invasive and non-pharmaceutical treatment options for peripheral arterial disease (PAD)

Non-invasive treatment options for PAD have been developed but are not fully adapted in clinical use [62]. The role and place for the following devices as primary treatment or an adjunct are not clear [62], although, according to Global Vascular guidelines on CLTI, intermittent pneumatic compression (IPC) should be considered to treat patients with CLTI and who are not eligible for revascularization, [57].

Intermittent pneumatic compression (IPC)

There are several devices for IPC therapy available [62]. The different devices deliver various pressure-settings, but all can rapidly and sequentially apply pressure to the calf or foot, or both, at the treated lower limb [63]. IPC increases blood flow in the popliteal artery and decrease venous pressure. A release for angiogenic growth factor and nitric oxide has been observed [63, 64]. The mechanism of action is suggested to be an increase in arterial flow due to increased arteriovenous pressure gradient and release of vasoactive substances, suspending the venoarterial reflex and stimulating growth of collateral arterials [57, 65]. A review evaluating IPC treatment for intermittent claudication, found an increase in maximum walking distance (MWD) with a mean

difference of 125 meter when compared to controls [63]. However, the overall quality of the included studies was regarded as poor, due to heterogeneity between studies, small sample size and low generalizability [63]. In a randomized controlled trial, including patients with critical ischemia with wounds not eligible for revascularization, an increase in MWD and faster ulcer-healing was found in the IPC-treatment group [64].

An IPC-device, arterial assist pump, produce intermittent venous occlusion, venous stasis and increase vein pressure, this in opposition to the above-mentioned devices. In a small study, 18 patients were treated for two years, without a control-group. An increase in walking distance by 20 - 120% and increase in toe-brachial index was found [66]. They presumed that suppressing the arteriolar vasoconstrictive activity by rhythmic and multiple obstructions of venous outflow led to a persistent capillary dilatation over time resulting in improved blood flow and increased walking capacity [64].

It might seem that IPC can reduce amputations in patients with CLTI [67], and it is suggested that IPC may be used in patient with CLTI in whom revascularization is not possible [57, 62].

Electrical Stimulators

Different transcutaneous electronic nerve and muscle stimulators exist [62, 68]. They operate at various settings to deliver an electrical impulse to the gastrocnemius muscle, or other related underlying muscles or nerves to evoke muscle contraction [62]. An increase in flow in the superficial femoral artery has been showed in a small cohort study. In studies comparing neuro-muscular electrical stimulation to a sham device, a significant increase in pain-free walking distance (PWD) and MWD was observed [62]. The mechanism of action is proposed to be an enhanced capillary supply blood flow and elevated oxidative enzyme capacity [68]. The studies have low sample size and high risk of bias, care should be taken to draw firm conclusion [68].

Extracorporeal shockwave therapy

Some small studies describe the use of external shockwave therapy, delivering acoustic energy to the calf muscle [69-71]. The potential mechanism of action is the expression of

angiogenic factors through the conversion of mechanical stimuli into chemical signals [70, 72]. External shockwave therapy may reduce vessel wall stenosis [70]. These effects lead to improved limb perfusion [69, 70] and a significant increase in PWD was seen in randomized controlled trial [71, 72].

Intermittent negative pressure (INP) treatment

INP applied to the lower extremities was introduced in the early 20th century as non-invasive treatment modality to improve peripheral blood circulation [73-75]. Additional effect of INP to standard care for patients with PAD, improving walking distance, have been suggested [76, 77]. Two recent randomized controlled studies found no additional effect [78, 79]. Both studies used INP treatment at low intensity for a short duration. Ranging from two to three times a week, for six weeks, with 30 minutes of INP treatment in total. This may have masked an eventual effect INP. In a study by Hoel et al (2020), alternating ten seconds of 40 mmHg negative pressure and seven seconds of atmospheric pressure was found to be the most effective INP treatment protocol acutely increasing blood flow [48, 49, 80]. With a treatment protocol of 40 mmHg INP for one hour twice a day, hard-to-heal wounds showed a tendency to heal [81, 82]. The beneficial effects of INP treatment is proposed to be caused by an acute increase in arterial blood flow [49, 80, 83], and fluctuations in shear pattern promoting endothelial function [84]. A qualitative study, evaluating patient and clinical experience and opinions of home use of FlowOx™, found that patients and clinicians were positive about the device and its ease of use [85].

Diagnosis, Monitoring and Surveillance of Peripheral Blood Circulation

To diagnose vascular disease, to plan treatment and to evaluate the results of an intervention, it is of importance to have a precise diagnostic toolbox. These have a range from clinical examinations, hand-held Doppler devices to advanced ultrasound-machines, as well as non-invasive and invasive radiological imaging with or without functional perfusion techniques and devices for evaluating microcirculation found in a

well-equipped vascular investigational laboratory. The use varies from daily to only in an experimental setting. In the following sections, a brief overview of the methods available, limited to monitoring and surveillance of PAD for diagnosis and per- and post-operative follow-up, are given.

Clinical examination

The initial evaluation of patients with suspected PAD often starts with a thorough clinical examination that includes palpation of pulses. Palpation of pulses at specific anatomic position of interest, like the abdominal aorta, radial, femoral, popliteal, posterior tibial and dorsal pedal arteries, is a screening tool. Diminishing of pulses indicate reduced circulation. If pulses are non-palpable, a handheld Doppler device, continuous wave Doppler (CW Doppler), should be used [50, 86]. Palpation of thrill at the outflow vein in an AVF is used as a clinical evaluation of the hemodialysis access [87-89]. Palpation of pulses postoperative are probably the most frequently used method to evaluate patency of the established revascularization.

Ankle-Brachial Index (ABI)

A useful bedside noninvasive test is assessment of peripheral blood pressure [86]. ABI is measured by placement of a sphygmomanometer at the proximal arms and ankles. Blood flow peripheral of the manometer is evaluated with CW Doppler. To calculate ABI, the highest measured Doppler systolic pressure at the tibial or dorsal pedal artery is divided by the highest pressure of the right or left brachial artery [90], equation 1. ABI should be measured separately on both legs.

$$ABI = \frac{\text{systolic ankle pressure (tibial of dorsal pedal artery)}}{\text{systolig arm pressure (right or left brachial artery)}} \quad (1)$$

An index between 0.9 and 1.3 is accepted as normal. An ABI below 0.9 is indicative of more than 50% stenosis and diagnostic for peripheral arterial disease, with a reasonably high specificity (83% – 99%) but lower sensitivity (69%–79%) [90]. Post-exercise ABI can be useful. ABI < 0.9 or an absolute fall of 30 mmHg when compared to the pre-exercise ABI, is suggested as significant [90]. Adverse outcomes after lower

extremity bypasses are associated with change in ABI. However, no incremental benefit of measurement of ABI to the clinical status was found [91]. ABI itself, cannot discriminate if a reduction in ABI is caused by a failure of the revascularization or progression of the underlying atherosclerotic disease [92].

Ultrasound (Spectral Doppler)

Vessel characteristics, included plaque and Doppler flow velocities, can be evaluated by sonography [93]. Ultrasound images (B-mode) are constructed from the reflected echo from sound waves propagated into tissue, generated by piezoelectrical element incorporated in a transducer [86]. Spatial and temporal resolution is set by pulse length, frequency and frame rate [94], which again is restricted by the physical properties, including the speed of sound and depth depicted. Absorption and reflection of the tissue reduce and weakens image quality [86].

Information about moving particles can be obtained using a Doppler technique, taking advantage of the change in frequency when the sound waves are reflected from a moving object (Doppler effect). This is used in CW Doppler, which transmit a continuous wave while a receiver continuous listens for echoes. CW Doppler does not discriminate depth of the source of the signals. In contrast pulsed wave (PW) Doppler transmit short burst of sound wave at a frequency, referred to as pulse repetition frequency (PRF), and listens to the echo signal with the same element. The time the soundwaves use to return (echo) corresponds to the specific depth [86]. The maximum Doppler frequency, which can be measured, is limited to half the PRF, named the Nyquist limit. Exceeding this limit results in aliasing [95].

PW Doppler measures movement towards or away from the probe. However, most often the vessel of interest lies parallel to the skin and the transducer. It is therefore fundamental to obtain adequate angle to the vessel of interest. Full Doppler shift is only obtained at 0° or 180°. The Doppler velocity (v) of the measured red blood cells is calculated by correcting for the insonation angle (θ), where the following is given c = speed of sound, $\int_e$ = emitted frequency, $\int_\Delta$ = Doppler frequency shift, equation 2.

$$v = \frac{c * \int_{\Delta}}{2 \int_{e} * \cos\theta} \quad (2)$$

Due the mathematical properties of the cosine function, an insonation angle close to perpendicular of the flow direction results in large difference in velocity caused by only a relatively small change in insonation angle. This increases the potential source of error of the measured Doppler velocity. An insonation angle <60° should therefore be achieved [86]. Color Doppler fuses a regular B-mode image with spectral Doppler as a color map, to show velocity within the B-mode imaging corresponding to velocity and direction [86].

Ultrasound is an applicable validated tool for assessing arterial and venous disease [96], as well as in control regimes after vascular surgery [97]. Sources of error includes physical limitations by the ultrasound technology, as well as anatomic and vascular properties that must be accounted for. Small structures and slow flow signals which overlap tissue movement, disturbs the evaluation of microvasculature. Dynamic filtering methods, termed superb vascular imaging (SMI) [95], use of contrast enhanced ultrasound (CEUS) [98-101] and high frequency ultrasound (20-200 MHz) [102] has been used to improve the visualization of the microcirculatory function.

Plethysmography

Plethysmography allows evaluation of PAD by three types of techniques: pulse-wave analysis, determination of digit of limb blood pressure and determination of arterial or venous blood flow. It records a change in dimensions of a portion of the body, as a reaction of temporarily obstruction of the venous system, expressed as volume. The technique is used to calculate arterial inflow [103].

Photoplethysmography records the change in reflection of the number of erythrocytes in the cutaneous circulation. Strain-gauge plethysmograph measures digit or limb volume through an elastic gauge and can obtain pulse volume waveforms. Air plethysmography measures changes in volume as momentary changes in pressure of

air-cuffs placed at point of interest. A variation of this technique is to place the whole limb in a chamber, and measure volume [86].

Pulsed wave trace analysis can determine the presence and degree of arterial occlusive disease. The digit and segmental limb systolic blood pressure can be determined by plethysmography and the plethysmography transducer can be used to detect the return of pulsatile flow following deflation of a pressure cuff, as used in some toe-pressure devices [86, 103].

Transcutaneous oxygen pressure ($TcPO_2$)

Quantitative estimation of cutaneous oxygen concentration in subcutaneous tissue one to two millimeters below the skin can be measured with a modification of the Clark polarographic oxygen electrode. Complete vasodilation is needed to eliminate variations in skin circulation, so the skin is heated between 43° and 45°. Oxygen metabolism, skin temperature, sympathetic activation, age, edema, hyperkeratosis, and cellulitis limits the accuracy of $TcPO_2$ [86, 104]. $TcPO_2$ can be used in screening for PAD [92, 105], although it is less able to discriminate between mild and severe disease [104]. $TcPO_2$ has been found most useful to predict wound healing and limb amputation in patients with diabetic foot ulcers with higher accuracy than ABI [106, 107].

Laser Doppler Flowmetry (LDF) and Laser Speckle Contrast Imaging (LSCI)

Laser Doppler Flowmetry (LDF) measures the reflected light from red blood cells moving in the skin microcirculation. The Doppler shift signal of the transmitted laser corresponds with the average velocity in the volume measured and is described as red blood cell flux [95]. LDF is easy to operate and can produce continuous measurements but cannot be given in absolute value and no calibration can be made. The reproducibility is therefore low and clinical widespread use is limited [108-111]. Systolic Skin Pressure (SSP) use LDF and a pressure cuff to measure the capillary opening pressure in a small area of the skin [86]. SPP-values >30 mmHg is associated with improved wound healing [92]. LDF to monitor and evaluate skin microcirculation, often in combination with a physiological test, can evaluate response on sympathetic

stimulation and endothelial function. LDF has been used to assess peripheral neuropathy and healing potential of wounds in diabetic patients [86].

By assessing multiple points over a broader area, a Laser Doppler Perfusion Image (LDPI) can be created. This technique reduce intra-measurement variability due to inhomogeneities [112] but do only give a static view of the perfusion. Laser Speckle Contrast Imaging (LSCI) combine continuous real-time imaging of LDF with LDPI and create a dynamic and readable perfusion map. A large area is often imaged, and therefore LSCI lacks the resolution for analysis of micro-vessels [95, 112].

Transcutaneous near-infrared spectroscopy (NIRS)

Near-infrared light (700-1000 nm) can penetrate the superficial layers (skin, fat, skull adipose tissue etc.) and is then either absorbed or scattered within the tissue. Changes in tissue oxygenation can be monitored through the variable absorption of near-infrared light by hemoglobin (Hb), myoglobin (Mb), and cytochrome c oxidase [113]. Transcutaneous near-infrared spectroscopy (NIRS) combines the oxygen saturation of venous and arterial blood, and is unable to differentiate between myoglobin (Mb) or hemoglobin (Hb), due to overlapping absorbency signal [113]. The latter influences the use of NIRS in monitoring oxygen saturation in peripheral tissue. Discrepancy exists of the clinical benefit for NIRS after revascularization [114]. NIRS is in clinical use for monitoring patients during carotid endarterectomy [115]. A systematic review found an estimated summarized sensitivity of 72.0% and specificity of 84.1%, compared to the awake test, and conclude that NIRS could not be the sole modality for detecting brain ischemia [116]. Further research is needed to define the role of intra-operative cerebral monitoring with NIRS.

Indocyanine green angiography

Tissue perfusion can be evaluated with indocyanine green (ICG) fluorescence angiography [117, 118]. ICG presents visual information of regional perfusion of the region of interest (e.g., foot) and is not limited of media sclerosis [119]. The degree of perfusion is quantified and can be used to predict wound healing [120]. ICG has been shown to be

feasible and safe and is a promising adjunct tool for diagnosing PAD and to predict postoperative outcome [119]. However, the use of ICG as a diagnostic tool and how to grade the degree of PAD, needs more research [114, 119].

Hyperspectral imaging (HSI) and Optical Coherence Tomography (OCT)

An anatomical map for tissue oxygenation can be constructed by using wavelengths between 500 and 660 nanometers of visual light. The light penetrates one to two millimeters of the skin. Absorption peaks for oxyhemoglobin and deoxyhemoglobin are measured. With hyperspectral imaging (HSI), no intravenous contrast is used, as in indocyanine green (ICG) fluorescence. Local hyperemic areas, like infections, will disturb the interpretations of the method [93].

Optical coherence tomography (OCT) is an imaging technique which can be compared to ultrasound, but instead of sound, creates images of the reflected light [121, 122]. It produces a cross-sectional image and 3D volume information [95, 121, 122]. OCT can detect microvascular flow due to the Doppler effect of reflected light [121, 123]. Novel devices do also combine HSI with OCT to create a functional and anatomic map of the area of interest. Although OCT is used to depict real-time vessel structure perioperative in endovascular crossing of an occlusive lesion, there is not enough evidence to suggest routine use [121].

Radiologic and Nuclear Imaging

Non-invasive radiologic imaging of peripheral circulation covers a broad array of imaging modalities e.g., computed tomographic (CT) angiography, magnetic resonance imaging (MRI) angiography, with or without functional perfusion techniques. These give an anatomic image of the vessels of interest and can, with the addition of perfusion techniques or by using paramagnetic properties of blood, quantify circulatory function [92]. The techniques are limited by errors caused by slow blood flow, often seen in PAD, and movement artifacts [92].

Nuclear imaging or radiotracer-based imaging can study various molecular processes to detect underlying pathophysiology in LEAD, in addition to its already established use

to evaluate osteomyelitis and vascular inflammation and/or infection [124]. Advancement in hybrid techniques, which fuse radiotracer imaging with high resolution CT or MRI, may assist in non-invasive serial monitoring of responses to treatment LEAD [124].

Capillaroscopy (intravital microscopy)

Reduced microcirculation can be evaluated by in vivo microscopic studies of skin capillary, usually in the nail skin folds. Dynamic or video capillaroscopy, with or without fluorescent dyes, can be used to assess microvascular dynamics, flow distribution and transcapillary diffusion [110, 125-130]. Capillaroscopy is mostly used in assessment of microcirculatory impairment in patients with Raynaud's phenomenon. Secondary complications of diabetes can be assessed, like reduced capillary density, microhemorrhages, and avascular areas. Capillaroscopy is also described in assessment of glaucoma and hypertensive patients. However, it is not widely used within assessing PAD [131]. The technique is cumbersome, requires well-perfused capillary network, special equipment, and trained examiners [95, 126, 130].

Clinical applications for earlybird

During the progress with this thesis several potential interesting clinical applications for earlybird were identified, adding to the original defined areas of early detection of diabetic autonomic dysfunction and peri-operative monitoring. In the following sections, a brief background for the clinical applications explored in the studies included in this thesis are given.

Diagnostic devices for evaluation of peripheral macro- and micro-circulation

There is a wide range of techniques and devices available for imaging and functional evaluation of peripheral macro- and microcirculation [86, 93, 95, 125, 126, 132-134], some are mentioned in the previous sections. Due to lack of standardization and their limitations, they are not widely adapted in a clinical setting [92].

Periprocedural monitoring during endovascular or open surgical lower limb revascularization

Standardized criteria to what extent needed to revascularize a lower limb to improve wound healing do not exist [135]. No objective method to periprocedural evaluate whether a limb is adequately revascularized are widely adapted in a clinical setting [114, 136]. Most studies have evaluated post-operative measurement to clinical outcome. Immediate post-operative palpation of distal pulses, hand-held Doppler evaluation and ABI pre- and post-operatively, are probably the most used objective assessment of the patency of a revascularized limb. An increase in ABI > 0.23 or TBI > 0.21 from before to after endovascular revascularization has been associated with wound healing [137]. An increase in ABI post-procedural 0.10 to 0.15 was found predictive for no residual stenosis [90]. TBI and ABI are not found as a dependable method for post-procedural evaluation, due to the variable diagnostic accuracy between studies and limitations of the methods due to calcified vessels or large wounds at the lower leg [92].

Earlier mentioned techniques for perioperative monitoring, like transcutaneous oxygen tension ($TcPO^2$) [138, 139], skin perfusion pressure (SPP), perfusion angiography, fluorescence angiography [120], tissue oxygen saturation mapping or duplex ultrasound (DUS) techniques with perioperative flow velocity measurements has been described to be of a potential benefit [57, 136]. Most of the techniques do not produce continuously flow evaluation in real-time or are not available peri-procedural [136]. Several tissue perfusion techniques are described; however, evidence remains low of diagnostic accuracy and related clinical outcome [92, 114].

During endovascular treatment, subjective evaluation of in-line flow, appearance of wound-blush, wash-out of contrast and improvement in vessel caliber are used to evaluate peri-procedural success [136]. Post-procedural measurement of the vascular flow reserve by an intraluminal thermodilution and pressure-sensor positioned in the popliteal artery, are associated with enhanced wound healing [140]. Open surgery allows for invasive monitoring. The global vascular guidelines on the management of CLTI recommends intraoperative imaging on completion of open bypass surgery. Significant

technical defects should result in immediate correction to improve patency and limb salvage [57]. Transit Time Flow Measurement (TTFM) consists of two small ultrasound transducer and a reflector, in a C-shaped probe. The difference in transit time between the reflected soundwaves of the downstream and upstream probes are used to calculate an objective measurement of VFR. The probes are produced at different diameters and can be placed perpendicular on the vessel of interest to intraoperatively evaluate graft flow [141]. TTFM is angle and diameter independent [142, 143], and found favorable in cardiac surgery [141], during creation of AVF for hemodialysis [144, 145] and lower limb bypass surgery [146-148].

Although many of the devices have shown feasible to detect changes in blood flow, circulatory or perfusion properties, they are not fully associated with clinical endpoints [114, 136]. Novel techniques like injectable oxygen micro-sensors implanted in the tissue of interest [149], laser-based real-time tissue perfusion device and hyperspectral imaging for superficial tissue oximetry are emerging and evaluated in clinical trials [92]. Continuously real-time evaluation of periprocedural hemodynamic or tissue perfusion parameters associated with clinical endpoint, like improvement in walking capacity and wound healing, could be a value tool to aid the clinician in the extent of revascularization.

Surveillance of volume flow in arteriovenous fistulas (AVF) for hemodialysis

Renal replacement therapy is the lifeline for patients with end-stage kidney disease. Establishment of an AVF for hemodialysis are preferred, but not the optimal vascular access. There is a high rate of primary mature failure. Primary patency rates at one and two years are 60% and 51% and secondary patency of 71% and 64%, respectively [150, 151]. To increase patency, surveillance of AVFs is suggested, but the benefit remains disputed [88, 152]. To detect clinical dysfunction of the vascular access it is recommended to perform regular clinical examination, supplemented by review of routine laboratory studies, evaluation of dialysis adequacy (urea reduction ratio and recirculation), cannulation difficulties and monitoring dynamic venous pressure [87]. When clinical dysfunction is detected, further evaluation and eventually intervention with open

surgery or endovascular intervention to improve patency should be made [87-89]. The rationale for surveillance strategies is to detect flow limiting stenosis and correct them before a thrombotic event occur [87]. Several methods exist for surveillance of AVF for hemodialysis, but the most used are measurements of access VFR, by ultrasound dilution method [89, 153, 154] or DUS [155-157]. DUS has the advantage that it can visualize functional and anatomic abnormalities [154]. ESVS guidelines promotes surveillance of AVF every third month and monthly for grafts, to reduce the risk of AVF-thrombosis [89]. However, updated guidelines from the European Renal Best Practice (ERBP) and The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) conclude that there is inadequate evidence to make a recommendation on AVF surveillance, and do not suggest routine surveillance of grafts [87, 88]. The included studies show a benefit for AVF surveillance regarding the rate of thrombosis and maybe a small benefit for AVF loss [88]. The guidelines have weighted this effect against the increase in interventions in the surveillance group [88]. If surveillance regimens are implemented in clinical practice, interventions should not be based solely on single VFR measurements. Trend-analysis and clinical dysfunction should be included in the evaluation [87-89]. Further research is needed to determine whether surveillance regimens with pre-emptive correction of a detected stenosis would reduce AVF loss or thrombosis, health economic analysis should be included [87, 88].

Aims of the thesis

1. Validate a novel ultrasound Doppler device, earlybird, against existing devices for assessing peripheral macro- and microcirculation – paper I.
2. To search for clinical applicability for earlybird within the broad area of vascular health, with focus on diagnostic, per- and postoperative monitoring and surveillance, and to evaluate earlybird's feasibility within the fields identified:
    a. Monitoring peripheral Doppler blood flow velocities and to evaluate flow characteristic, during and as response to treatment with INP (FlowOx™) in patients with PAD – paper III.
    b. Per-operative monitoring during endovascular treatment – congress abstract I.
    c. Surveillance of VFR in AVF for hemodialysis – paper IV.
3. Evaluate the effect of INP-therapy with FlowOx™ on walking capacity – paper II.

Methods

Four studies were conducted to evaluate earlybird's potential to detect and monitor peripheral vascular blood flow according to the revised areas of interest, table 1.

- Study I: Validation study to compare earlybird to existing methods for evaluating peripheral circulation and microcirculation, (paper I).
- Study II: To further gain experience with earlybird as a monitoring device and its potential, a randomized controlled study on the effects of treatment with INP on walking capacity in patients with intermittent claudication due to PAD was included (paper II). The feasibility of earlybird to monitor acute, and long-term circulatory changes as response to treatment with INP was studied (paper III).
- Study III: Evaluation of earlybird's ability to detect changes in peripheral blood flow velocity during endovascular surgery (congress abstract I)
- Study IV: Evaluation of the earlybird's ability to monitor VFR in AVF (paper IV)

Setting

Technological and software development, test subjects and patient enrollment were made in cooperation between Section of Vascular Surgery, Department of Surgery at St. Olav's Hospital, Department of Circulation and Medical Imaging, NTNU, Section of Vascular Investigations at Department of Vascular Surgery, Oslo University Hospital, and Department of Surgery at Sørlandet Hospital Kristiansand.

For study I, study subjects were recruited at Section of Vascular Investigations at the Oslo University hospital. In study II, a multicenter randomized trial, patients were included from all three clinical centers (Trondheim, Kristiansand, and Oslo). Study III tested earlybird during endovascular treatment at Sørlandet Hospital, Kristiansand. All patients enrolled in study IV were recruited from the dialysis department at Sørlandet

Hospital. The experimental part of study IV was set up at the facilities of the Department of Circulation and Medical Imaging, NTNU.

Table 1: Study-overview

Study	Study design	Participants (n)	Setting	Compared devices	Endpoint
I – paper I	Validation	10	Vascular Investigation Laboratory	Earlybird, LDF, PW Doppler	Correlation
II – paper II	RCT primary outcomes	63	Home-use	FlowOx™	Walking distance
II – paper III	RCT secondary outcomes	62	Home-use	Earlybird	Flow characteristics
III – congress abstract I	Pilot	1	Angio-suite	Earlybird	NA
IV – paper IV	Feasibility	16	Experimental laboratory and dialysis department	Earlybird DUS	Correlation

RCT, randomized controlled trial; LDF laser Doppler flowmetry; PW Doppler, pulsed wave Doppler; DUS, duplex ultrasound; n, number; NA, not applicable

Cohorts – test subjects and patients

For study I (paper I), ten healthy subjects were enrolled. They were monitored with PW Doppler, LDF, and earlybird for assessing microcirculation during physiological provocation tests, which elicited vasoconstriction or dilatation.

For study II (paper II and III), patients with established intermittent claudication due to PAD were recruited from three vascular surgery departments in Norway, St.Olavs Hospital, Trondheim, Oslo university hospital, Oslo, and Sørlandet Hospital, Kristiansand. Inclusion criteria were ABI < 0.9 in rest, or a presentation with

incompressible arteries combined with a radiological confirmed diagnosis of occlusive disease. Patients should not have been scheduled to or have undergone endovascular or open surgical revascularization within the previous three months. Exclusion criteria were inability to operate the treatment device independently, presence of severe heart disease or chronic obstructive pulmonary disease, and a MWD of more than 1000 meter measured on a treadmill.

For the pilot on perioperative monitoring presented in study III (congress abstract I), one patient undergoing endovascular treatment for intermittent claudication was recruited.

In addition to an experimental setup, patients with radio-cephalic forearm AVF established in hemodialysis were recruited for the study IV (paper IV). Patients with other types of AVF like grafts, brachio-cephalic or basilic vein fistulas were excluded. In total 16 patients were included.

Data collection

For all studies, simple background data was of interest. We collected information of age, weight, medical history, smoking habits, and tobacco and caffeine-use by interviewing the participants in study I. For patients in the clinical studies background information was partly collected through interview or from extracting the information of interest from the electronic patient journal.

Earlybird

A flat circular version of the earlybird 8 MHz probe (Imasonic SAS, Besançon, France) was used for study I. For study II, III and IV, a rectangular 8 MHz earlybird probe (Per Kristian Bolstad, University of Southeast Norway) fixed in a case providing approximate 60° insonation angle to the surface, was used. In-house software, developed in MATLAB (MathWorks ®R2016/R2018a/R2020b), recorded multi-gated Doppler signals

for real-time evaluation and later post processing of power-Doppler M-mode and spectrogram.

Placement of earlybird and additional measurements

Study I: Earlybird was placed above the digital artery of the first finger. Arterial blood flow velocity (cm/s) in the radial artery was measured with a 10 MHz PW Doppler probe (SD-50; GE Vingmed Ultrasound, Horten, Norway). Skin pulp blood flow was measured with LDF (Periflux PF 4000; Perimed AB, Jarfalla, Sweden). These data were simultaneously recorded at 1000 Hz in LabChart (ADInstruments, Dunedin, New Zealand).

Study II: Earlybird was placed at the dorsal pedal or posterior tibial artery. The same artery was chosen for both the test before and after 12 weeks of INP-treatment. Resting and post-exercise ABI and strain-gauge plethysmography (Domed Filtrass Angio, Krailling, Germany) was measured. To assess PWD and MWD, the patient underwent a treadmill test, using a ramp protocol at a constant speed of 3.2 km/h starting at 0% slope and increasing the slope by 2% every 2 minutes [158]. EQ-5D-5L [159]. Vascuqol-6 quality of life questionnaires [160] at baseline and after 12-weeks of treatment were completed by the patients.

Study III: Earlybird was placed at the posterior tibial artery at ankle. No additional functional test, evaluation of clinical outcome or peripheral circulation, excepts the already mentioned measurements with earlybird, were made.

Study IV: Earlybird was positioned above the phantom vessel of interest in the experimental setup, and at the anatomic location of the outflow vein of the AVF in the clinical setting. For the experimental setup of the study a precise measurement of the amount of fluid pushed through the closed circuit was made by weighing the variation of fluid in the reservoir during a given time. DUS VFR (ml/min) measurements were undertaken both for the experimental setting (GE Vivid E95 (General Electric (GE) Vingmed Ultrasound, Horten, Norway)), and clinical setup (GE Logic S8 ultrasound system (GE Healthcare, Milwaukee, Wisconsin, USA)).

Physiological stress-tests, INP treatment, and endovascular intervention

Study I: Different physiological stress responses to elicit vaso-constriction or dilatation were undertaken; forced respiration, isometric handgrip exercise, Valsalva maneuver and cold pressor test.

Study II: Each participant was randomized to a 12-week treatment with either 40 mmHg INP (active-boot) or 10 mmHg INP (sham-boot) treatment for one hour, twice daily.

Study III: The patient underwent a therapeutic endovascular treatment with crossing of an occlusion of the external iliac artery, pre-dilatation, stent placement and post-dilatation.

Study IV: No intervention was made.

Statistics

All data was plotted or analyzed in Microsoft Excel, SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY), Stata, version 16 (StataCorp, College Station, Tex) or MATLAB (MathWorks ® R2016, R2018a and R2020b). Figures were produced with SigmaPlot (Systat Software, San Jose, CA) and SPSS, figures and images were optimized for presentation in Adobe Photoshop Version: 22.4.2 (Adobe, San Jose, CA).

Descriptive statistics are presented as the median with its 25th and 75th percentile, mean with its 95% confidence intervals or standard deviation for continuous data, numbers with percentages for categorical variables. Normality was assessed by histograms, Q-Q plots, residual plots and Shapiro-Wilk test. Students t-test or Mann-Whitney U test were used for comparison between groups. Paired sampled t-test or One-Sample Wilcoxon Signed Rank were used for analyzing within groups differences for continuous variables. Differences in distribution or between groups were analyzed with χ^2 test for of categorical variables and Kruskal-Wallis H for continuous data. For

post hoc analysis, Mann-Whitney U for pairwise comparison with Holm-Bonferroni correction were used. Pearson or Spearman rank correlation were used for correlation analyses. Zero-mean normalized cross-correlation (ZNCC) were used for analysis of LDF, PW Doppler and earlybird velocity curves. Agreement between methods and intra-rater variability were analyzed with intraclass correlation coefficient (ICC). Bland-Altman plots with 95% limits of agreement and mean-square-root was used to analyze variability and accuracy of the methods for measuring volume flow. P-values of < 0.05 were regarded as statistically significant.

Funding

A PhD research grant was awarded Erik Mulder Pettersen from NTNU Innovation, at the Faculty of Medicine and Health Sciences.

Ethics

Norwegian Government of Health (June 12th, 2017) approved earlybird as an investigational device, but restricted it use to observational studies, not allowing treatment to be altered by the information gathered by earlybird.

The Regional Committee for Medical and Health Research Ethics in Norway (reference no. 2017/044) approved the studies regarding the validation and clinical applicability of earlybird (study I, III and IV)

The INP-study (Study II) was approved by the Regional Committee for Medical and Health Research Ethics in Norway (reference no. 2018/748) and was registered at ClinicalTrials.gov (identifier: NCT03640676).

Written informed consent was obtained from all study-subjects and patients before inclusion.

Otivio and Cimon Medical was not involved in the study design; collection, analysis, or interpretation of data; manuscript writing; or the decision to submit the manuscript for publication.

Summary of studies

Study I, Paper I: Validation of a novel ultrasound Doppler monitoring device (earlybird) for detection of microvascular circulatory changes.

Background: A range of non-invasive methods are available to assess microcirculation and peripheral blood flow [95, 104, 105, 126, 133, 161]. In this study earlybird was validated against PW Doppler and LDF.

Method: Ten healthy volunteers were recruited. On the right hand and fingers several simultaneous recordings were done. Mean arterial blood flow velocity (cm/s) of the radial artery was measured with a 10 MHz pulsed PW Doppler probe (SD-50; GE Vingmed Ultrasound, Horten, Norway), and skin pulp blood flow was measured with LDF (Periflux PF 4000; Perimed AB, Jarfalla, Sweden). All data were assessed simultaneously and recorded at 1000 Hz in LabChart (ADInstruments, Dunedin, New Zealand). Earlybird was placed above a small digital artery at the first finger to measure blood flow velocity. In each subject we recorded a five-minute baseline recording and four different protocols; forced respiration, isometric handgrip exercise, Valsalva maneuver and cold pressor test. Each protocol was repeated twice. The data was normalized against the mean of the whole recording for baseline and the first 25 seconds for the provocation tests. Zero-mean normalized cross correlation (ZNCC) was used to evaluate correlation between earlybird, LDF and PW Doppler, respectively.

Results: In total 90 paired measurements were made. For earlybird and LDF 76 paired measurements, and for earlybird and PW Doppler 82 paired measurements were available for further analysis. Excluded measurements were due to malfunction, inappropriate placed probes, or missing recordings. For both baseline and provocation tests the blood flow and velocity curves recorded by the different devices are in visual concordance. ZNCC shows an overall high correlation, with a median correlation of all measurements of 0.90 (25th - 75th percentile 0.82 – 0.95) for earlybird and PW Doppler and 0.87 (0.77 – 0.91) for earlybird and LDF measurements, figure 5. ZNCC

analysis for the provocation test for earlybird and LDF, and earlybird and PW Doppler, shows respectively: forced respiration: 0.87 (0.28 – 0.90), 0.90 (0.85 – 0.96), static handgrip: 0.82 (0.59 – 0.90), 0.87 (0.68 – 0.94), Valsalva maneuver: 0.88 (0.82 – 0.91), 0.94 (0,92 – 0,97) and cold pressor: 0.90 (0.85 – 0.95) 0.89 (0.65 – 0.94).

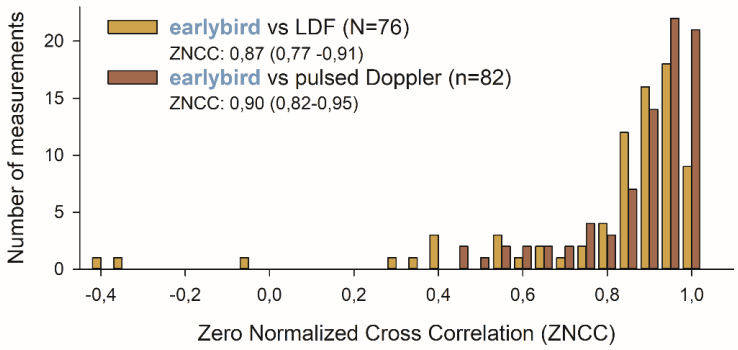


Figure 5: Histogram of all recordings, of Zero Normalized Cross Correlation of earlybird versus laser Doppler flowmetry (LDF) and pulsed wave Doppler, respectively.

Conclusion: Earlybird records vasoconstrictions in healthy subjects as well as LDF and pulsed Doppler. Earlybird may be a future tool to assess peripheral circulation.

Study II, Paper II: A randomized controlled trial of treatment with intermittent negative pressure (INP) for intermittent claudication.

Background: A proportion of the patients with stable intermittent claudication due to PAD are not suitable for first-line treatment with exercise programs. [59-61]. A wide range of non-invasive non-pharmaceutical treatment options have emerged as an adjunct or alternative treatment [62]. INP applied to the lower body or extremities have been described to improve blood flow and increase walking distance in patients with PAD [48, 49, 76, 77, 80]. The aim of this study was to investigate the clinical effects of treatment with 40 mm Hg INP, for 12 weeks in patients with intermittent claudication.

Method: In a multicenter randomized double blinded sham-controlled study 85 patients with stable intermittent claudication due to PAD were found eligible, 72 patients were included. A total of 63 patients were available for follow up and intention-to-treat analysis. The patients were randomized to either treatment with 40 mmHg INP or sham-treatment with 10 mmHg INP. The INP treatment was applied using a special designed pressure chamber (boot) connected to a pump unit (FlowOx™, Otivio AS, Oslo, Norway), image 3. The patients were trained to treat themselves at home, with one hour at the morning and one hour in the evening for 12-weeks. Patients were tested at baseline and after 12 weeks of INP treatment, with treadmill test, ABI at rest and post-treadmill-test, and strain plethysmography. EQ-5D-5L and VascuQOL-6, quality of life questionnaires, were completed before and after the 12-week INP treatment period.

Results: Baseline characteristics were not different between the groups except that the proportion of patients with diabetes mellitus were larger in the treatment group. After 12-week INP treatment the 40 mmHg INP treatment group had significant longer PWD, mean treatment effect 50 meters (95% CI 11 - 89), $p = 0.014$. In the treatment group, compared to baseline, PWD had increased with 68 meters (95% CI 33-103), $p < 0.001$, and MWD 62 m (95% CI 19-105), $p = 0.006$. For the 10 mmHg INP group, PWD and MWD did not significantly increase, mean change 18 meters (95% CI -1 to 38), $p = 0.139$, and 20 meters (95% CI -16 to 57), $p = 0.265$, respectively. Between the 40 mmHg

and 10 mmHg INP treatment groups no significant difference was found for the change in MWD after 12 weeks of INP treatment, mean difference 42 meters (95% CI -14 to 97), p = 0.14. No significant changes were found for resting or post exercise ABI across the groups (p = 0.65 and p = 0.19). No significant changes in resting or post-ischemic blood flow, measured with plethysmography, was observed across the groups (p = 0.34 and p = 0.58). No significant change in quality-of-life questionnaires were observed across the groups (EQ-5D-5L, p = 0.67, EQ-5D-5L visual analog score p = 0.29, VascuQOL-6, p = 0.89). A sub-analysis of patients with PWD < 200 meters (n = 56) at baseline, showed a significant difference between the treatment groups for both PWD and MWD in favor of the 40mmgH INP treatment group, estimated treatment effect PWD = 42 meter (95% CI 2-83), p = 0.041 and MWD = 62 meters (95% CI 5-118), p = 0.032.

Conclusion: In patients with intermittent claudication due to PAD, 12 weeks of INP treatment with 40 mmHg one hour, twice daily, seem to increase PWD, when compared to patients treated with a sham device providing 10 mmHg INP. For patients with a baseline PWD < 200 m and increase in both PWD and MWD in the treatment group was found, when compared to the sham-group.

Study II, Paper III: The effect of 12-week treatment with intermittent negative pressure on blood flow velocity and flowmotion, measured with a novel Doppler device (earlybird). Data from a randomized controlled trial in patients with peripheral arterial disease.

Background: Treatment with INP is proposed as an adjunct to standard care in PAD [162, 163]. However, all patients included in the INP-studies did not improve walking capacity [163]. The aim of this study was to evaluate the applicability of earlybird to assess blood flow characteristics in patients with PAD during a treatment session with INP, and whether certain flow-properties could determine whom could benefit INP treatment.

Method: In this study, secondary outcomes from the earlier published randomized sham-controlled multicenter trial on the clinical effect of 12-weeks 40 mmHg INP treatment, one hour twice daily, in patients with intermittent claudication, was explored [162]. For details on recruitment and inclusion of patients see summary paper II. Blood flow velocity measurements were recorded with earlybird before and after the 12-week INP treatment period. Earlybird was attached to tibial posterior or dorsal pedal artery at the leg treated. A 5-minute recording in rest were made. After a short break another recording was undertaken, consisting of 1-minute recording before INP test-treatment, a 3-minute recording during INP test-treatment, and a 5-minute recording after the INP test-treatment was stopped. All recordings were saved for post processing. Mean Doppler velocity (v_{mean}) was used for further analysis. Power spectrum analysis by Fourier-transform was applied to the Doppler blood flow velocity-curves to analyze different characteristics of flowmotion and INP-response. The area under the power spectrum curve was calculated for each frequency band corresponding to specific flow motion characteristics; 0.007 to 0.02 Hz , 0.02 to 0.06 Hz, 0.06 to 0.2 Hz, 0.2 to 0.6 Hz and 0.6 to 1.8 Hz associated to endothelial, sympathetic, myogenic, respiratory and heart activity, respectively [164-166].

Results: Data was available for 62 patients, where 30 patients were treated with 10mHg INP and 32 patients with 40 mmHg INP. Earlybird recordings were of good quality and eligible for further analyses.

We confirmed earlier findings [48, 49, 80], that v_{mean} increase during the negative pressure periods of the INP test-treatment. The difference between the medians of relative increase in v_{mean}, in patients treated with 40 mmHg compared to 10 mmHg, were 13.7 ($p < 0.001$) and 10.7 ($p < 0.001$) at pre- and post-test respectively. Spectrum analysis of the bandwidth associated to INP treatment confirmed this finding ($p < 0.001$) for both pre- and posttest.

A difference between the treatment groups was found for the change from pre- to posttest for the normalized amplitude corresponding to myogenic function (0.06 – 0.2 Hz), difference between the medians 0.86, $p = 0.002$. The median change for 10 mmHg INP-group was -0.99 (-4.0, 0.01), $p = 0.003$ and 40 mmHg INP treatment-group was 0.85 (-1.3, 2.6), $p = 0.167$. The myogenic function is mainly caused by activity in the smooth muscles of arteriolar vasculature [167]. The finding may indicate that INP recruit arterioles and thereby improve peripheral blood circulation.

An extensive explorative analysis of the available data was done. Changes in flow velocity, INP-response or different aspects of flow motion was not found to associate with categories of PWD and MWD or improvement in PWD and MWD. No correlation was found between relative change in v_{mean} and flowmotion variables and improvement in PWD or MWD. Thorough scrutinization did not reveal flow characteristics that could predict whom could benefit INP treatment.

Conclusion: Earlybird is an applicable tool for assessing blood flow velocity in patients with PAD. The acquired velocity recordings showed that INP induce an immediate increase in blood flow velocities during INP. The positive effects of INP may be attributed to recruitment of arterioles, and thereby increasing blood flow. In these analyses no flow characteristics was determined which could predict whom would benefit INP treatment. But the study was neither designed, nor provided an adequate sample size to properly evaluate these possible research questions.

Study III, Congress Abstract I: Earlybird - A Novel Ultrasound Doppler Monitoring Device - Potential Future Application in Per-Operative Monitoring

Background: CLTI is the end stage of PAD and requires prompt and adequate revascularization to prevent limb loss [57]. No objective methods to evaluate whether a limb is adequately revascularized are widely adopted in a clinical setting [136]. Determination of periprocedural noninvasive hemodynamic parameters could guide the extent of anatomic revascularization and on-site clinical decision making [92, 168]. Future potential of Earlybird could be per-operative monitoring to assess adequate limb revascularization. To determine whether Earlybird could detect change in blood velocities during an endovascular procedure a pilot study was performed.

Methods: During an endovascular procedure an occlusion of the external iliac artery was passed with a catheter and predilated. A stent was placed, followed by balloon dilation. An Earlybird probe was attached over the posterior tibial artery at the ankle of the treated limb. Per-operative flow velocities were recorded. The recorded data was processed to evaluate change in v_{mean}.

Results: At baseline, v_{mean} was 4.58 cm/s. No increase in v_{mean} was found at predilation or at stentplacement. First after ballon dilation of the stent an increase in v_{mean} to 7.81 cm/s was seen (increase from baseline: 3.23 cm/s (171 %)), figure 6.

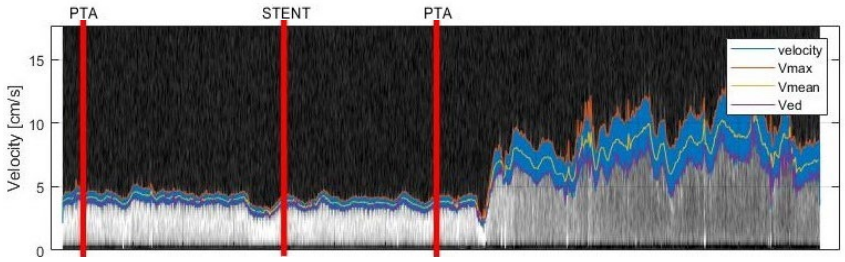


Figure 6: Mean blood flow velocity (v_{mean})(cm/s) during endovascular treatment.

Conclusion: Earlybird detects changes in flow velocities during an endovascular revascularization procedure. Further investigations are needed to assess hemodynamic properties associated with clinical outcome. Earlybird could be a future tool for clinical decision making during endovascular treatment.

Study IV, Paper IV: Validation of a novel ultrasound Doppler monitoring device (earlybird) for measurements of volume flow rate (VFR) in arteriovenous fistulas for hemodialysis.

Background: Regular surveillance of VFR is suggested to preserve the limited number of available vascular accesses for hemodialysis in patients with terminal kidney failure [89]. Controversy exists about the benefit of surveillance [87, 88]. Trend-analysis has been suggested to overcome methodological limitations and low reproducibility [87, 89]. In this study we examined whether earlybird could be a future device for surveillance of VFR.

Method: In an experimental and clinical setting we compared earlybird to DUS. In a setup which consisted of a rotator pump, venting reservoir, scale, tubing, and peripheral vascular phantom, 36 paired recordings of earlybird, DUS and calibrated VFR were measured. These recordings included simulated vessel diameter of four-, six- and eight-millimeter at different calibrated VFR. Calibrated VFR was measured by the amount of water moved through the system in 60 seconds, measured with a scale and assuming one liter is equal to one kilogram. In 16 patients, 23 paired recordings of VFR with earlybird and DUS were made at the contralateral and ipsilateral brachial artery, radial artery, and the AVF outflow vein.

Results were analyzed with Pearson correlation (r) and intraclass correlation coefficient (ICC), as well as Bland-Altman plots and root-mean-square (RMS), which were calculated based on the relative difference of DUS and earlybird from the calibrated VFR for the experimental data, and the relative difference from the mean of DUS and earlybird VFR at the AVF outflow vein for the clinical setup.

Results: In the experimental setup, it was found that earlybird VFR had a strong correlation, excellent agreement, and good accuracy, compared to calibrated VFR, r = 0.991 ($p < 0.001$), ICC = 0.970 (95% CI, 0.932 - 0.985), RMS = 15,6%, while for DUS VFR, r = 0.984 ($p < 0.001$), ICC = 0.949 (95% CI, 0.449 - 0.986) and RMS = 36.3%. In the Bland-Altman plot the constant bias for the relative difference for earlybird VFR was -10.5% ((SE 1.96), $p < 0.001$), 95% limits for -33.5 and 12.6%, and for DUS VFR of 27.2% ((SE

4.06) p <0.001), 95% limits of -20.5 and 74.9%. Proportional bias for earlybird VFR was B = 0.023 (p <0.001) and for DUS VFR was B = -0.029 (p = 0.017), figure 7.

In the clinical setup, we found that earlybird, compared to DUS VFR, had a strong correlation, good agreement and accuracy, r = 0.781 (p <0.001), ICC = 0.750 ((95% CI 0.502, 0.885), p <0.001) and RMS = 16.2%. In the Bland-Altmann plot, no significant relative difference from the mean VFR for the two methods was found, 2.9% ((SE 3.39), p = 0.399), 95% limits -29.0 and 34.8%. No proportional bias was detected (p = 0.245).

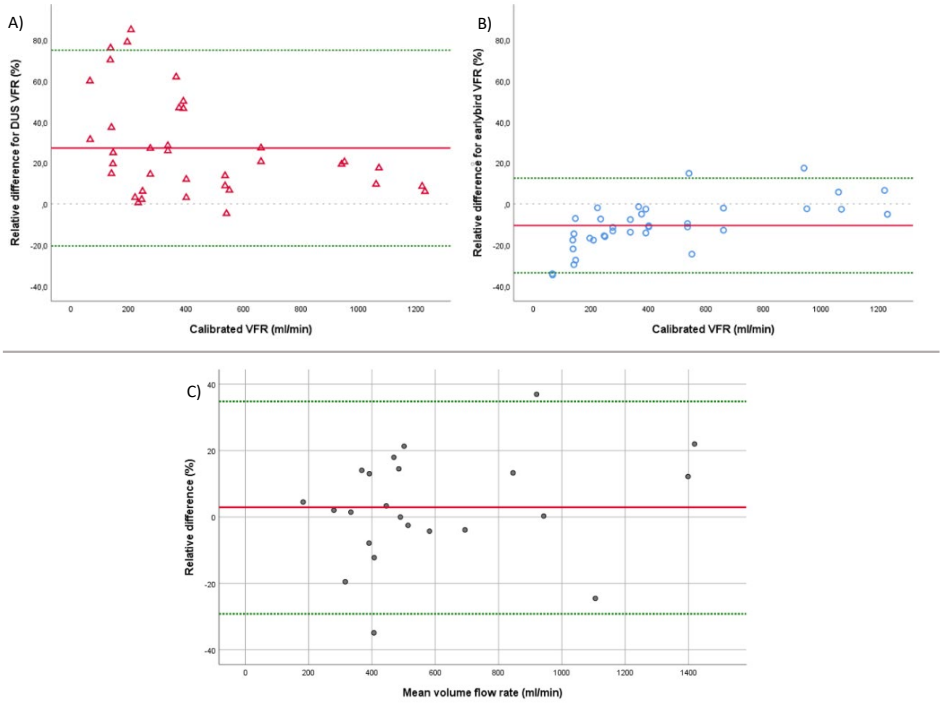


Figure 7: Bland-Altman plots: A) and B): Relative difference (%) of duplex ultrasound (DUS) or earlybird, and the calibrated volume flow rate (VFR) ((VFR—calibrated VFR)/calibrated VFR). C: Relative difference (%) of DUS and earlybird VFR from the mean VFR ((earlybird VFR—mean VFR)/mean VFR), measured at AVF outflow vein. Mean difference (red solid line) and 95% limits of agreement (dotted green line).

Conclusion: Earlybird is a feasible tool for evaluating VFR in AVFs. Technical development may overcome the major limitation of angle-dependency, increase user-friendliness, and further improve the accuracy. Earlybird could be a future device for surveillance of VFR to be used for trend-analysis.

General discussion of results

Two possible innovations within the field of peripheral vascular diseases were investigated in this thesis. Earlybird is a promising novel ultrasound Doppler device for monitoring peripheral blood flow. FlowOx ™ is a treatment-device providing INP within a pressure chamber applied to the lower extremity and improves blood flow to the extremities and walking ability in patients with peripheral atherosclerotic disease.

In four studies earlybird's feasibility to monitor peripheral arterial blood flow velocity was evaluated. Specific clinical applicability within the panorama of peripheral vascular diseases has been explored. Earlybird was able to measure blood flow during ongoing INP treatment. A pilot study showed that earlybird can monitor an immediate change in circulation due to successful endovascular intervention in real-time. Earlybird is also shown to be able to monitor, with an acceptable accuracy, volume flow in AVFs in patients established for hemodialysis. Further research and development of earlybird is needed to increase user-friendliness, its clinical applicability and to determine cut-off values and standardize measurements.

In a randomized sham-controlled study, it was found that 12-week treatment with INP increase PWD when compared to sham-treatment. Analyses of the recorded flow velocities found that INP elicits an acute increase in blood flow. At 12 weeks of treatment a difference in change in myogenic function between the treatment groups were found. This could indicate that arterioles may be involved in the positive effects shown with INP treatment. Further investigations are needed to determine this finding. INP treatment, as delivered by FlowOx™ may be supplementary to existing treatment of patients with PAD, through its positive effect on peripheral blood circulation.

What does earlybird add?

In this thesis, an exploration of possible clinical applications of earlybird as a diagnostic monitor and surveillance tool for peripheral arterial circulation were conducted.

A novel device for monitoring peripheral circulation

The transducer of earlybird is a highly sensitive single element probe with a wide acoustic beam equivalent the size of the probe [34, 46]. Information of blood flow velocities is gathered by using PW Doppler technique, simultaneously and continuously for the chosen interval for the whole preset depth. This is in contrast to existing PW Doppler devices, where a more accurate placement and depth determination is needed [169].

Other devices as $TcPO_2$, plethysmographs or LDF are potentially expensive equipment, need experienced personnel and are therefore less available. Although these devices have been associated with useful clinical endpoints, the main barrier for widespread implementation is the lack of standardization [45, 110, 133, 170, 171]. The same limitations are valid for earlybird. Earlybird has the potential to be produced as a small easy-to-use handheld portable device [46]. This may facilitate data collection in proper conducted studies to assess flow characteristics, and determine hemodynamic properties associated with clinical endpoints.

In the studies conducted and included in this thesis, earlybird was found to be a feasible tool for the measurement of blood flow velocities and providing data of good quality for post processing and analysis. Earlybird and the PW Doppler-probe strongly correlated in study I. Both devices use PW Doppler to acquire information about the Doppler shift, and measure blood flow velocities at a digital and radial artery, respectively. LDF, on the other hand, measures flux from the Doppler shift of red blood cell in a small given volume of the pulp skin [86, 95]. In study II and III, earlybird was the only device used for measuring blood circulation, hence no comparison with other devices could be done. However, during ongoing INP test-treatment sequence in patients with PAD, reliable flow velocities were recorded and available for analyses. To accurate measure absolute blood flow velocity values, the precise insonation angle to the vessel of interest is needed to make a proper angle correction of the measured velocities [86]. However, this is not known for earlybird, and is a major limitation of the device. Thus, only the relative change in flow, can be used to evaluate peripheral blood

flow characteristics. This limitation also applies to many of the other devices used to assess peripheral circulation e.g., single PW Doppler probe and LDF [86, 133]. For NIRS, the relative measured change in saturation from baseline is most validated for clinical use [115, 116, 172]. Tissue perfusion imaging techniques, such as $TcPO_2$, systolic skin pressure (SSP), and indocyanine green (ICG) do provide an absolute measurement of tissue oxygen pressure, perfusion, or circulation [114]. DUS, with angle-corrected PW Doppler measurement, do provide absolute and correct velocity measurement and allow determining cut-off values for clinical outcome through follow up in time [86, 97]. To further explore earlybird as a tool to assess relative change as response to a provocation test to evoke a vasomotor response or to develop earlybird to be angle independent could increase its feasibility and clinical applicability.

Earlybird is a potential advantageous device to assess peripheral blood circulation. Due to its large and highly sensitive transducer and unique feature to monitor blood flow velocity simultaneously and continuously at several depths, it is easy to place and need no depth determination. Earlybird has the potential to be used by non-specialized health care personnel, lay-persons, and patients without extensive training, in a wide range of clinical applications. However, as technical development will take place, hemodynamic properties associated with clinical endpoints and feasibility should be affirmed.

Perioperative monitoring

Long-term patency after endovascular or open surgical revascularization varies [57]. Interventions on distal arteries in the calf are associated with lower patency than more proximal lesions [57]. In CLTI, limited time is available to assess the degree of revascularization, due to the risk of developing irreversible gangrene. Determination of periprocedural noninvasive hemodynamic parameters to guide the extent of anatomic revascularization and on-site decision making are needed to reduce procedural-time, perioperative risks, increase patency and to secure limb salvage [92, 168].

Establishment of in-line flow, appearance of “wound blush”, flow characteristics and improvement of arterial calibers are used to subjective evaluate peri-procedural success [136]. Post-operative non-invasive tests has been associated to wound healing and clinical improvement [92, 136]. Other studies have suggested that periprocedural monitoring with 2D perfusion angiography [173-177], fluorescence angiography [119, 120, 178], $TcPO_2$ [179], NIRS [172], skin perfusion pressure (SPP) [180, 181], and duplex ultrasound can be of potential benefit [136]. These techniques or devices are not widely used in clinical practice due to lack of standardization and validated cut-off values [136]. Perfusion angiography can quantify change in perfusion parameters. It uses a specific protocol with the existing angio-suite through ad-on software, but the method is very sensitive of limb movements [173-177]. $TcPO_2$ detect local increase in tissue oxygen perfusion. Change in $TcPO_2$-levels are in most studies, done pre- and post-operative and do then not give real-time periprocedural information of perfusion levels [182]. In a recent trial in patient with in-line revascularization to the foot, no peri-procedural increase in $TcPO_2$ was found. An increase was shown on postoperative follow up, while toe-pressure increased immediately after the endovascular intervention [138]. Skin perfusion pressure has also been shown to increase up to one month after endovascular treatment [183]. It may seem that an improvement in measured tissue perfusion is delayed, and that the method is less suitable for real-time periprocedural monitoring.

In the pilot study of monitoring peripheral blood circulation during endovascular treatment, the feasibility of the earlybird was demonstrated. Earlybird can assess changes in blood flow in real-time as response to endovascular treatment. In the published pilot only experience for one patient was presented. An immediate increase in Doppler velocities was seen after successful revascularization. Whether this finding or other hemodynamic properties measured with earlybird can predict clinical improvement and wound healing should be evaluated in further studies with more patients included.

Volume flow rate (VFR) surveillance in arteriovenous fistulas for hemodialysis

VFR surveillance of vascular accesses for hemodialysis are suggested to increase long-term patency of AVF [89] and reduce the transition to central venous catheter, which is associated with an increased rate of bacteremia and decreased survival [87]. Meta-analyses of the role of VFR surveillance in AVF for hemodialysis has shown that surveillance reduce thrombosis and loss of fistula, but the same benefit is not seen for AV-graft [157, 184]. The benefit of surveillance regimens remains uncertain, partly because higher intervention rate and post-intervention thrombosis with loss of patency are seen in the surveillance-groups [87-89]. VFR trend analysis is suggested to improve surveillance regimens and could redefine treatment algorithms, but further evaluation is needed [87, 89].

We found that earlybird is a feasible tool for monitoring VFR in AVFs. In an experimental setting earlybird seem more accurate than DUS. This may be explained by the use of clutter-filter in DUS, which filters signal from slow moving tissue and vessel wall, often with a high intensity, and thereby cause a relative higher VFR at low VFR-values. It is assumed that the vessel of interest lies parallel to the skin surface, but the AVF outflow vein may deviate. Earlybird do not visualize the course of the vessel. This is one of the greatest limitations of earlybird, especially in a clinical setting, while not present to same degree in the experimental setting since the phantom vessel lays parallel to the surface. VFR measurements at the AVF outflow vein are also influenced by vessel tortuosity and diameter variations, which may reduce the full development of laminar parabolic flow. The diameter calculation of earlybird is based on M-mode and Doppler spectrogram, deviations in the calculated diameter will also reduce the accuracy of the calculated volume flow rate. Technical development of the earlybird probe-system and software can increase the accuracy.

Bouthier et al (1983) described an angle independent pulsed wave Doppler-system, including two probes, to monitor VFR in AVFs [185]. Further development of an angle independent monitoring device could increase earlybirds feasibility and provide higher

accuracy of the measurements, an extend its use to other clinical applications like circulatory assessment and intraoperative monitoring.

What does FlowOx™ add?

A randomized sham-controlled trial of 12 weeks treatment with 40 mmHg INP was included in this thesis. The study showed that INP treatment induce an increase in PWD compared to sham-treatment, as well as increase in MWD for patients with a PWD < 200 meters [162]. In a follow up study of ten patients, treated for 24 weeks with INP, not included in this thesis, both an increase in PWD and MWD was found when compared to baseline [163]. Recently an economic model to examine cost-effectiveness of FlowOx™, compared to standard care in PAD suggested that the treatment may be cost-effective [186]. Earlier performed randomized controlled studies, which did not show a benefit of INP, used treatment protocols with lower intensity of 30 minutes of INP treatment ranging from two to three times a week for six weeks in total [78, 79]. It may seem that INP treatment with higher intensity for a longer duration is favorable and improves walking capacity in patients with intermittent claudication due to arterial occlusive disease.

The mechanism of action of INP treatment is suggested to be an increased arteriovenous gradient [49, 80, 81, 83, 187], as well as fluctuations in shear-pattern increasing endothelial function [84]. In study III the immediate increase in blood flow velocity induced by INP treatment found in earlier studies [49], was confirmed. The study also found a difference between the treatment groups in change in myogenic function. In an exploratory study on the same group of patients, a reduction in von Willebrand factor (vWF) was observed within the 40 mmHg INP treatment group. This was not observed in the 10 mmHg INP treatment group [188]. These findings may indicate a beneficial effect of INP treatment on vasomotor muscle cell activity, endothelial activation, and endothelial injury. There may be a possible effect of flow mediated dilation as response to INP treatment, and it indicates a potential field of interest which could be evaluated further in a proper conducted study.

In study II, paper III, in addition to the feasibility of earlybird, flow properties induced by INP treatment was studied. There are several limitations to this study, in terms of interpretation and assessment of current findings, or lack thereof. The study was designed to evaluate the effect of INP treatment on walking distance, and not for evaluating blood flow characteristics.

Changes in blood flow velocities was evaluated as response of a 3-minute INP test-treatment, which is not a validated physiological test to evaluate vasomotor response. As a consequence of the double-blinded nature of the study, and not to unblind the study-participants, only 32 of in total 62 study participants underwent a test-treatment with 40 mmHg INP before the 12-week INP treatment. The rest underwent a test-treatment with 10 mmHg INP which was not expected to induce a significant vasomotor response [48]. This lowers the power to study differences between the groups and change in flow properties during the 12-week INP treatment.

A 5-minute recording of both treatment-groups were recorded in rest. Spectrum analyses by Fourier-transform, were used to evaluate flowmotion associated to endothelial, sympathetic, and myogenic activity. Due to the long waves of the associated endothelial activity (0.007 – 0.02 Hz), only one to two cycles are included in the 5-minute recorded sample. Ideally the recording should have sampled blood flow velocities for 15 to 20 minutes, to fully be able to evaluate flowmotion.

Changes in blood flow velocities and flowmotion characteristics were evaluated against improvement in walking capacity. Although, 12 weeks of INP treatment resulted in an increase in PWD and MWD, only a significant difference between the 10 and 40 mmHg INP treatment groups was found for the increase in PWD. The estimated treatment effect was 50 meters. The above-mentioned factors reduce the study II's potential to evaluate flow characteristics and to eventually determine factors who could select patients who are more likely to benefit INP treatment.

Supervised exercise therapy (SET) is recommended as the first-line treatment for patients with intermittent claudication [50]. There is strong evidence of the effectiveness

and efficiency of SET [50, 189]. However, INP treatment could be an adjunct to SET or be considered for patients not eligible for SET. The role of INP treatment in patients with intermittent claudication due to PAD should be further clarified.

Non-invasive treatments for patients with severe or critical limb ischemia, e.g., IPC, may reduce the risk of amputations [190]. Treatment with IPC is associated with limb salvage, wound healing, and improved walking distance [191]. Although, the studies included in systematic reviews had a high risk of bias [190, 191]. Global vascular guidelines on management of critical threatening limb ischemia (CLTI) recommend that in patients with rest pain and minor tissue loss, who are not eligible for revascularization, IPC should be considered as a treatment option [57]. Complications of IPC-treatment include pain, contact rash and abrasion of the skin due to the repetitive compression [191]. In study II, no serious adverse effects of FlowOx™ were observed, neither was this reported by other studies examining INP treatment. This could be a benefit for patients with CLTI, who often have vulnerable skin. The mechanism of action of INP treatment share some of the characteristics with IPC [57, 65]. With the recent evidence, INP treatment may have a role in patients with CLTI in whom revascularization is not possible.

Earlybird and FlowOx™ in an innovational context

FlowOx™ by Otivio is established as a health technology company. Flowox™ is in use by first-users and has established sales program to distribute their product [47, 192]. During the process of the thesis, Cimon Medical was established to develop and promote the underlying technology of earlybird [46]. The two devices are in different stages of the innovation process. FlowOx™ is on the verge of expanding its clinical use to the early adopters (stage 8 - 9 in the healthcare innovation cycle), while the technology behind earlybird has undergone a range of feasibility and initial clinical trials (stage 6 in the healthcare innovation cycle) [22]. Close co-operation with the intermediate and end-user is established in clinical networks to determine potential areas of application and to evaluate clinical outcome.

Introduction of new technological treatment devices or diagnostic tools into clinical practice raises challenging ethical issues involving evaluation of evidence, balancing benefits and harms, supporting patient autonomy, avoiding conflict of interest, and promoting advances in health care [193]. The medical consequences and traditional ethical aspects should be balanced in light of the complex and plural sociocultural values [194]. A review by Hofmann (2013) identified the main challenges with emerging welfare technologies as: 1) "valienation when advanced technology is used at home", 2) "conflicting goals, as welfare technologies have many stakeholders with several ends", 3) "respecting confidentiality and privacy when third party actors are involved", 4) "guaranteeing equal access and just distribution", and 5) "handling conflicts between instrumental rationality and care in terms of respecting dignity and vulnerability" [195]. To systematically evaluate properties, effects and/or impacts of healthcare technology and address their intended as well as unintended consequences, Health Technology Assessment (HTA) organizations and agencies were introduced. These State mechanisms are intended to preserve the balance between the benefit of new technology and the risks and costs [196, 197]. According to the European Network of Health Technology Assessment (EUnetHTA), HTA is defined as ''a multidisciplinary process that summarizes information about the medical, social, economic and ethical issues related to the use of a health technology in a systematic, transparent, unbiased, robust manner" [198]. In Norway, new methods for diagnostic, and treatment, including pharmaceuticals, are considered through the methodology of "Nye Metoder" in cooperation with The Norwegian Medicines Agency and The Norwegian Institute of Public Health [199]. A peer-reviewed mini-HTA was undertaken for INP-therapy (mini-metodevurdering, April 2021) [200]. No decisive conclusion has yet been made. However, the reviewer commented that the role of INP treatment for patients with intermittent claudication (IC) is unclear, but there seems to be a large gain for patients with critical threatening limb ischemia, especially in those who are not eligible for revascularization [201].

The interest of society and expectation of healthcare towards new advances within medicine stimulates clinicians and academia to increasing interaction with industry [202, 203]. Medical innovation depends on, and emerge from, this interplay and can generate funding and increase diffusion of new technology and insight [203, 204]. The increased push towards commercialization need consideration, and the following risks has been mentioned; 1) "impacts on research practices", goal, trends and sharing of results (open science), 2) "science hype" by overstatement of research results and exaggerated publicity, 3) "premature implementation", 4) public trust may decrease due to ties to industry, 5) "skewed health policy" toward economic generating innovation and away from socio-economic and behavioral change and prophylactic medicine, and 6) "damage to the long-term economic contributions of university research", due to focus on near-term translational activities, and a shift away from ideas that require basic research [203]. Several factors may improve the university-industrial collaboration positively; regulatory, earlier experience and personal relations, transfer of knowledge through industry-funded PhDs, as well as temporarily hiring a researcher and transfer of research results, and geographical or spatial closeness [202].

FlowOx™ and earlybird are part of costly and time-consuming development process. When beneficial effects of an innovation are identified, effective path to commercialization (diffusion) should be sought. Barriers of innovation can be defined as the same as the critical success factors, but with opposite sign; regulation, investor risk, cumbersome development process and excessive pricing [13]. A recent rapport from SINTEF, commissioned by InnoMed, showed that the four main barriers for effective implementation of new solutions in the municipal and health sector is: 1) "the economic room for maneuver", 2) "culture of change", 3) "anchoring", and 4) "legislation" [205]. However, the SARS-CoV-2 (Covid) pandemic has shown that many of the barriers can be broken, by close collaboration between clinicians, academia, healthcare decisionmakers and industry partners [206, 207]. The universities have established technical transfer offices to give regulatory advice, protect patents and

intellectual property (IP), and to facilitate the commercialization and innovation-process [29, 30]. Tension may exist between the researchers' interest in early publication and the technical transfer offices' interest of securing patent and IP rights [30]. It is argued for that technical transfer offices should have less focus on patent and IP rights, but rather be a tool for the universities to put the research to use for the greater good of our society [30].

This thesis incorporates the clinical applicability of two innovational devices and has not explored the formal and unformal role of university-industry collaboration, although such interaction exists for both devices. However, several of the above-mentioned factors have probably been crucial to the development of both earlybird and FlowOx™. Close collaboration and personal relations exist between academia and Otiovio, and part of the underlying research have been through industry funded PhDs. Earlybird's underlying technology is developed in an academic environment (Department of Medical Imaging, NTNU) which share office facilities with industry and are located in the same building as the clinical activity. The close collaboration is probably vital for development of both FlowOx™ and earlybird. It enhances the clinical applicability, through frequently intersection with intermediate and end-users. On the other hand, needed regulatory limitations, may delay development. Earlybird is not CE-marked, and research is restricted to observational studies (Norwegian Government of Health, June 12th, 2017). Experimental trials are necessary to explore the diagnostic parameters and determine useful and valid clinical endpoints [208]. This thesis can illustrate how the scientific evolvement often follows stages of the innovation process; through validation of the concept (paper I), feasibility of the device in a diseased population (paper III), pilot studies to gain experience of possible clinical applications (congress abstract I), validation of clinical application (paper IV) and estimation of effect and clinical outcome (paper II). The scientific research provides evidence of the degree of benefit of the innovation.

Interaction between clinician and researcher, together with patient involvement are needed to develop modern health care. Cooperation with other stakeholders,

municipals, hospital administration and industry, is equally necessary to secure the innovation process, through implementation and commercialization. Emilie Wise (2006) concluded: “Yet regardless of whether innovation is driven by price, technology or user needs, the end goal is the same: that consumers pay for it, companies make a profit, national welfare and competitive strength increases. So the common denominator is the same – a focus on meeting consumer needs.”[20]

Development of healthcare services, devices and treatments are needed to overcome the challenges in healthcare of today. Improvement in medical practice through innovation, should be done in interaction and cooperation with patients, clinicians, academia, legislators, and industry. Healthcare innovations may to a large degree affect our daily life. The consequences should undergo thorough ethical consideration together with scrutinization of the cost-benefit.

Conclusion

In this thesis, two innovational devices were studied: a novel Duplex ultrasound device for monitoring peripheral circulation and a treatment device providing INP to increase peripheral blood circulation to the lower limbs. Both innovation and commercialization projects originate from technology environments. Through scientific research benefits of the innovation are sought, to fulfill society demands of cost-effective solutions to the benefit of the patient.

Earlybird is an applicable tool for assessing blood flow velocity in healthy individuals and in patients with peripheral arterial disease. The acquired flow velocity recordings were eligible for analyses of flow characteristics and flowmotion. Earlybird is a feasible tool for monitoring VFR in AVF in patients established in hemodialysis, and it may be a future device for VFR surveillance and allow for trend-monitoring. Earlybird is an applicable tool to detect changes in blood flow velocities as a response to endovascular treatment, but further research should be made to establish eventually cut-off values associated to clinical outcome. Further technological development could increase user-friendliness and clinical applications.

Treatment with INP, increase walking capacity and peripheral blood flow. FlowOx™ could be a supplement to standard care of intermittent claudication in patients with PAD. INP treatment may be a useful treatment device in patients with CLTI without irreversible gangrene, whom are not candidates for revascularization.

Perspectives and future possibilities

Development of earlybird could allow for a user-friendly low-cost device for both evaluation of peripheral circulation and post-operative surveillance after vascular intervention.

Further exploration of clinical applicability and technical development is needed. During the innovation project of earlybird, software extensions have been developed that automatically can extract measurements of interest. These, as well as not yet identified functionality, could be further developed to increase user-friendliness and accuracy as well as clinical applicability. The device is currently able to measure relative change in blood flow velocities. One of the major limitations is earlybirds angle dependency to assess exact Doppler velocities. Potential future development could be implementing and testing of an angle independent measurement of Doppler velocity. To fully take advantage of the potential for peri-operative monitoring to aid clinical decision making, one must specify clinical endpoints in relation to hemodynamic parameters, through observational and interventional prospective studies. The experience from the study on VFR could be adapted to other areas within the field of vascular health. Further research is needed to determine the benefit of VFR surveillance in AVFs and/or grafts. Prospective observational studies of the relation of VFR and vascular access adverse events could further evaluate the applicability of earlybird. Randomized controlled trials are necessary to evaluate an eventual benefit of a surveillance algorithm which includes earlybird.

Future potential areas of interest, which was part of the original project in an early phase, would be evaluation of the detection of autonomic dysfunction, e.g., diabetes mellitus. Prospective longitudinal studies could be undertaken to evaluate adverse events and detection rate to establish potential relation between an autonomic dysfunction, detected by earlybird, and clinical endpoints. Doppler technology could be incorporated in continuously in-vivo graft surveillance, industry involvement and in-

vitro and animal testing, could evaluate the potential for further clinical applicability. Although pilot-studies of similar products are published [209]. Comparison of earlybird to other novel hand-held, smartphone based devices could be of interest [210].

Studies on treatment with INP treatment, with FlowOx™, has gathered evidence of a potential benefit of the device to improve walking capacity in patients with peripheral atherosclerotic disease (PAD). However, a clarification of the role of INP treatment is needed. It is appropriate to conduct a follow-up RCT to study the population of interest. Possible applications of INP treatment could be: 1) adjunct to exercise treatment, 2) adjunct to endovascular treatment, and 3) treatment option for patients not eligible to standard of care treatment (e.g., revascularization or exercise treatment). Further studies should also evaluate treatment duration and intensity.

Both earlybird and FlowOx™ are dependent on interest of the users (or market), and not at least investors willingness to fund further development. Willingness to pay, public reimbursement and regulations will affect the possibility to commercialize these two devices. FlowOx™ is already commercially available. Assessment of the end-user needs, barriers of implementation as well as development of a sound business plan, especially for earlybird, are natural steps of a further commercialization process.

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Paper I

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Validation of a novel ultrasound Doppler monitoring device (earlybird) for detection of microvascular circulatory changes

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Abstract.
OBJECTIVE: In this proof-of-concept study we aim to validate a novel ultrasound Doppler monitoring device for evaluating microcirculation (earlybird) against LDF and pulsed Doppler.
METHODS: In ten healthy subjects, we measured microcirculatory function at rest and during different autonomic tests (forced respiration, isometric exercise, Valsalva maneuver and cold pressor). Earlybird, LDF and pulsed Doppler were recorded simultaneously. We performed a ZNCC to determine correlation.
RESULTS: The curves for earlybird and LDF or pulsed Doppler correlates visually well. Overall median ZNCC 0.87 (interquartile range 0.77 – 0.91) between the LDF and earlybird measurements, and 0.90 (0.82 – 0.95) for pulsed Doppler and earlybird. Median ZNCC for baseline and each provocation test for earlybird against LDF and pulsed Doppler were calculated; baseline: LDF 0.87 (0.73 – 0.97) pulsed Doppler 0.91 (0.81 – 0.94), forced respiration: LDF 0.87 (0.28 – 0.90) pulsed Doppler 0.90 (0.85 – 0.96), isometric exercise: LDF 0.82 (0.59 – 0.90) pulsed Doppler 0.87 (0.68 – 0.94), Valsalva maneuver: LDF 0.88 (0.82 – 0.91) pulsed Doppler 0.94 (0.92 – 0.97) and cold pressor: LDF 0.90 (0.85 – 0.95) pulsed Doppler 0.89 (0.65 – 0.94).
CONCLUSION: Earlybird records vasoconstrictions in healthy subjects as well as LDF and pulsed Doppler.

Keywords: Ultrasonography, Doppler, Laser-Doppler Flowmetry, peripheral arterial disease, inventions, microcirculation

Abbreviations

LDF Laser Doppler flowmetry
ZNCC Zero-normalized-cross-correlation

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Paper II

A randomized controlled trial of treatment with intermittent negative pressure for intermittent claudication

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ABSTRACT

Objective: We investigated the effects of lower extremity intermittent negative pressure (INP) treatment for 1 hour two times daily for 12 weeks on the walking distance of patients with intermittent claudication (IC).

Methods: Patients with IC were randomized to treatment with −40 mm Hg INP (treatment group) or −10 mm Hg INP (sham control group). Pain-free walking distance (PWD) and maximal walking distance (MWD) on a treadmill, resting and postexercise ankle-brachial index, resting and postischemic blood flow (plethysmography), and quality of life (EQ-5D-5L and Vascuqol-6) were measured at baseline and after 12 weeks of treatment.

Results: A total of 72 patients were randomized, and 63 had data available for the intention-to-treat analyses. The between-group comparisons showed a significant change in the PWD, favoring the treatment group over the sham control group (estimated treatment effect, 50 m; 95% confidence interval [CI], 11-89; $P = .014$). The PWD had increased by 68 m ($P < .001$) in the treatment group and 18 m ($P = .064$) in the sham control group. No significant difference was found in the change in the MWD between the two groups (estimated treatment effect, 42 m; 95% CI, −14 to 97; $P = .139$). The MWD had increased by 62 m ($P = .006$) in the treatment group and 20 m ($P = .265$) in the sham control group. For patients with a baseline PWD of <200 m (n = 56), significant changes had occurred in both PWD and MWD between the two groups, favoring the treatment group (estimated treatment effect, 42 m; 95% CI, 2-83; $P = .042$; and estimated treatment effect, 62 m; 95% CI, 5-118; $P = .032$; respectively). Both overall and for the group of patients with a PWD <200 m, no significant differences were found in the changes in the resting and postexercise ankle-brachial index, resting and postischemic blood flow, or quality of life parameters between the two groups.

Conclusions: Treatment with −40 mm Hg INP increased the PWD compared with sham treatment in patients with IC. For the patients with a baseline PWD of <200 m, an increase was found in both PWD and MWD compared with sham treatment. (J Vasc Surg 2021;73:1750-8.)

Keywords: Intermittent claudication; Intermittent negative pressure treatment; Peripheral artery disease

Peripheral artery disease (PAD) affects >235 million people globally, and the prevalence is increasing.[1] Intermittent claudication (IC) is a common symptom in patients with PAD characterized by muscle discomfort in the lower limb that is provoked by exercise and relieved by rest[2] and is associated with reduced ambulatory activity and quality of life.[3,4] Participation in supervised exercise therapy (SET) programs increase the walking capacity of patients with IC[5,6] and is the first-line treatment, together with smoking cessation and pharmacologic secondary prevention.[7] However, the availability of SET programs is low,[8] and many patients are unwilling or unable to participate.[9] Consequently, home-based exercise programs and different treatment devices have been suggested as alternative treatment options.[10-12]

Methods using intermittent negative pressure (INP) applied to the lower body or extremities to improve

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Author conflict of interest: H.H. is employed by Otivio AS with funding from The Research Council of Norway (grant 285758). I.M. is the chief strategy officer and shareholder in Otivio AS. Otivio AS has the commercial rights to the intermittent negative pressure technology used in the present study. E.M.P., LØ.H., A.S., and J.H. have no conflicts of interests. Otivio AS was not involved in the study design; collection, analysis, or interpretation of data; manuscript writing; or the decision to submit the manuscript for publication.

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blood flow in patients with PAD have been described since the early 20th century.[13-15] Several studies have suggested positive effects on walking distance in patients with IC.[16-18] However, two recent studies did not find any additional effects of INP treatment on walking capacity.[19,20] Because the treatment intensity and duration varies among previous studies and the results differ, the clinical effect of INP treatment for patients with IC remains uncertain.

The aim of the present study was to investigate the clinical effects of treatment with −40 mm Hg INP for 1 hour in the morning and 1 hour in the evening for 12 weeks in patients with IC. We hypothesized that this treatment would improve the pain-free walking distance (PWD) and maximal walking distance (MWD) compared with sham treatment.

ARTICLE HIGHLIGHTS

- **Type of Research:** A multicenter, prospective, randomized controlled trial
- **Key Findings:** Treatment of intermittent claudication with lower extremity intermittent negative pressure for 1 hour twice daily for 12 weeks increased the pain-free walking distance in the treatment group (n = 38) receiving −40 mm Hg intermittent negative pressure compared with the sham control group (n = 34) receiving −10 mm Hg intermittent negative pressure.
- **Take Home Message:** Treatment with lower extremity intermittent negative pressure increased the pain-free walking distance compared with sham treatment for patients with intermittent claudication. For the patients with the most symptomatic disease, an increase occurred in both pain-free and maximal walking distance compared with sham treatment.

METHODS

Participants. We performed a multicenter trial, enrolling patients from the outpatient clinics at three vascular surgery departments in Norway (Oslo University Hospital, Oslo; Sørlandet Hospital, Kristiansand; and St Olavs Hospital, Trondheim) from January to September 2019. Data collection was completed in December 2019. Patients with an ankle-brachial index (ABI) of ≤0.9 or incompressible arteries and a radiologic diagnosis of PAD and IC were assessed for eligibility. Patients who had been scheduled for or who had undergone endovascular or open surgical revascularization within the previous 3 months were considered ineligible. The exclusion criteria were: inability to provide an informed consent; inability to perform a treadmill test; inability to independently operate the treatment device; the presence of severe heart disease; the presence of severe chronic obstructive pulmonary disease; and a baseline MWD of >1000 m measured on a treadmill with a ramp protocol.[21] All patients were offered the best medical treatment according to the guidelines from the European Society of Cardiology and the European Society for Vascular Surgery.[7]

INP treatment. INP was applied in a pressure chamber sealed around the lower leg. A pump unit (FlowOx 2.0; Otivio AS, Oslo, Norway) removed air from, and vented, the pressure chamber (Fig 1), producing alternating 10 seconds negative pressure and 7 seconds atmospheric pressure. The patients in the treatment group received −40 mm Hg INP, and the sham control group received −10 mm Hg INP. The devices were otherwise identical. These INP levels were chosen according to the findings from a recent study from our research group demonstrating that −40 mm Hg INP induced an acute increase in blood flow in the treated extremity in patients with PAD, in contrast to −10 mm Hg INP which did not significantly affect blood flow.[22]

All patients were instructed to treat themselves at home for 1 hour in the morning and 1 hour in the evening for 12 weeks. They were trained in the use of the INP device before the start of treatment. The daily treatment time was recorded by the device, allowing for the analysis of compliance data after the intervention period. To avoid the direct effects of treatment on the test results, the patients were instructed not to use the device on the day of the 12-week follow-up examination.

Randomization and blinding. Patients were randomized to the treatment group or sham control group in a 1:1 ratio using a computer-generated randomization list. Labeling of the treatment devices was performed by the producer (Otivio AS) by a person not involved in patient recruitment or data collection. The patients and personnel with patient contact during the study period were unaware of the group allocation. The statistical analyses were also performed without knowledge of the treatment group.

Clinical evaluation and measurements. Clinical evaluation and measurements were performed by the same person at baseline and after 12 weeks of treatment. The primary outcome measures were the changes in PWD and MWD. The pain-free walking time and maximal walking time were measured with the patients walking on a treadmill using a ramp protocol at a constant speed of 3.2 km/h starting at a 0% slope and increasing the slope by 2% every 2 minutes.[21] The patients were asked to specify the most limiting leg after the baseline treadmill test, which was chosen as the treatment leg.

Before the treadmill test, the resting ABI was measured with the patient in a supine position according to the guidelines from the American Heart Association.[23] The

Fig 1. Device for lower extremity intermittent negative pressure (INP) treatment. INP is generated in a pressure chamber sealed around the patient's lower leg by a pump unit that removes air from, and vents, the pressure chamber. Provided by Otivio AS, Oslo, Norway.

postexercise ABI was measured with the patient in supine position within 1 minute after the end of the treadmill test.

Resting blood flow was measured with the patient in the supine position using strain-gauge plethysmography (Domed Filtrass Angio, Krailling, Germany) of the lower leg at the point of maximal circumference. The plethysmograph records the rate of change in volume expansion for the area under the strain-gauge during proximal venous occlusion on the thigh, and the blood flow values are calculated.[24] Postischemic blood flow was measured using the same strain-gauge plethysmograph after 3 minutes of arterial occlusion obtained by inflating the thigh cuff to 250 mm Hg. For safety reasons, patients who had previously undergone bypass surgery in the leg were excluded from the present examination, as were patients who experienced unbearable pain during occlusion.

All the patients were requested to complete the EQ-5D-5L and Vascuqol-6 quality of life questionnaires at baseline and after 12 weeks of treatment. The EQ-5D-5L questionnaire consists of a visual analog scale and a descriptive system.[25] Using each patient's score in the descriptive system, an index value was calculated (EQ-5D-5L index) based on a value set validated for Denmark.[26] The Vascuqol-6 is a health-related quality of life questionnaire for patients with PAD validated for Norway,[27] consisting of six disease-specific items with a total score ranging from 6 to 24, with a higher score indicating better health.

Statistical analysis. The data are presented as the mean ± standard deviation for continuous variables and numbers and percentages for categorical variables, unless otherwise stated. Normality was assessed by histograms, Q-Q plots, and residual plots. The baseline characteristics between the groups were compared using independent samples *t*-tests for continuous variables and χ^2 tests for categorical variables. Differences within the groups were analyzed using paired sampled *t*-tests. Differences between groups were evaluated using univariate analysis of covariance, adjusting for differences in baseline data.[28] All subjects with pre- and post-treatment data were included in the intention-to-treat analyses. *P* values ≤.05 were considered statistically significant. Analyses were performed using Stata, version 16 (StataCorp, College Station, Tex).

In a comparable population of patients with IC, the pain-free walking time on a treadmill was 146 ± 112 seconds.[29] Assuming an increase in pain-free walking time of 87 seconds (76 m) as a clinically important difference,[30] 26 patients per treatment arm were required to detect a treatment effect, given 80% power and a 5% significance level. Assuming a withdrawal rate of 25%, we aimed to include 35 patients in each group.

Ethics. The Regional Committee for Medical and Health Research Ethics in Norway approved the present study (reference no. 2018/748), which was registered at ClinicalTrials.gov (identifier, NCT03640676). All the patients provided written informed consent before inclusion.

RESULTS

A total of 85 patients were assessed for eligibility. Of the 85 patients, 2 did not meet the inclusion criteria (baseline MWD >1000 m), and 11 patients declined to participate, leaving 72 patients (85%) for randomization (Oslo University Hospital, n = 46; St Olavs Hospital, n = 5; Sørlandet Hospital, n = 21; Fig 2). At baseline, a significantly higher prevalence of diabetes was present in the treatment group compared with the sham control group (*P* = .008). No significant differences were found between the two groups for all other demographic variables (Table I).

Walking distance. The between-group comparisons showed a significant difference in the change in the PWD, favoring the treatment group compared with the sham control group (estimated treatment effect, 50 m; 95% confidence interval [CI], 11-89; *P* = .014; Table II). At baseline, the PWD was 109 ± 70 m in the treatment group and 111 ± 77 m in the sham control group (Table I). After 12 weeks of treatment, an increase in the PWD of 68 m had occurred in the treatment group (95% CI, 33-103; *P* < .001) and 18 m in the sham control group (95% CI, −1 to 38; *P* = .064; Fig 3).

The between-group comparisons showed no significant differences in the change in the MWD after 12 weeks of INP treatment (estimated treatment effect, 42 m; 95% CI, −14 to 97; *P* = .14; Table II). The baseline MWD was 267 ± 177 m in the treatment group and 263 ± 170 m in the sham control group (Table I). After 12 weeks of

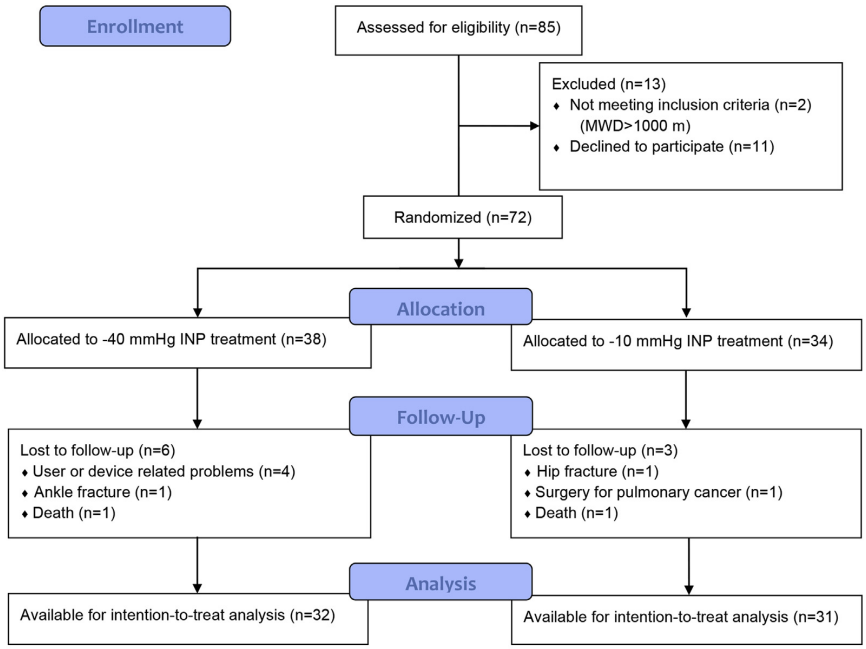

Fig 2. CONSORT (consolidated standards of reporting trials) flow diagram showing inclusion, exclusion, treatment allocation, and outcomes. *INP*, Intermittent negative pressure; *MWD*, maximal walking distance.

treatment, an increase had occurred in the MWD of 62 m in the treatment group (95% CI, 19-105; $P = .006$) and 20 m in the sham control group (95% CI, −16 to 57; $P = .27$; Fig 3).

Of the 63 patients who had completed the 12-week intervention period, 56 (89%) had had a baseline PWD of <200 m. For these patients, the between-group comparisons showed statistically significant differences in the changes in both PWD and MWD, favoring the treatment group (estimated treatment effect, 42 m; 95% CI, 2-83; $P = .042$; and estimated treatment effect, 62 m; 95% CI, 5-118; $P = .032$, respectively; Table III).

ABI, blood flow measurements, and quality of life. At baseline, the resting ABI was 0.53 ± 0.16 in the treatment group and 0.56 ± 0.15 in the sham control group (Table I). No significant changes were found in the resting or postexercise ABIs across the groups ($P = .65$ and $P = .19$, respectively), and the plethysmography measurements did not show significant changes in the resting or post-ischemic blood flow across the groups ($P = .34$ and $P = .58$, respectively) after 12 weeks of treatment. Furthermore, no significant changes were observed in the quality of life questionnaire scores (EQ-5D-5L index, $P = .67$; EQ-5D-5L visual analog scale score, $P = .29$; Vascuqol-6, $P = .89$) across the groups after 12 weeks of treatment (Table II). Relative within-group changes for all outcome variables after 12 weeks of treatment are illustrated in the Supplementary Fig (online only).

Compliance, discontinuation, and adverse events. The mean daily treatment time was 1.8 ± 0.2 hours in the treatment group and 1.8 ± 0.5 hours in the sham control group ($P = .63$). Six patients in the treatment group and three patients in the sham control group were lost to follow-up (Fig 2). Four patients in the treatment group discontinued treatment because of issues related to use of the treatment device. One patient in the treatment group and one patient in the sham control group discontinued because of severe trauma (ankle fracture and hip fracture, respectively), and one patient in the sham control group discontinued because of surgery for pulmonary cancer. One patient in each group died, both of cardiac arrest (unrelated to the INP sessions) during the 12-week intervention period. No further serious adverse events were reported.

DISCUSSION

The main finding from the present study was that treatment with −40 mm Hg INP for 1 hour in the morning and 1 hour in the evening for 12 weeks increased the PWD compared with sham treatment for patients with IC. For the patients with a baseline PWD of <200 m

Table I. Baseline patient characteristics

Variable	Treatment group (n = 38)	Sham control group (n = 34)	*P* value
Age, years	72 ± 8	73 ± 6	.59[a]
Male sex	25 (66)	26 (76)	.32[b]
Body mass index, kg/m^2	27.3 ± 4.2	26.9 ± 4.0	.74[a]
Smoking			.92[b]
Current	14 (37)	11 (32)	
Previous	19 (50)	18 (53)	
Never	15 (39)	5 (15)	
Comorbidity			
Diabetes mellitus	18 (47)	6 (18)	.008[b]
Chronic renal failure	5 (13)	4 (12)	.86[b]
Hypertension	32 (84)	28 (82)	.83[b]
Hypercholesterolemia	22 (58)	27 (79)	.051[b]
Coronary artery disease	17 (45)	18 (53)	.49[b]
Cerebrovascular disease	8 (21)	8 (24)	.80[b]
Antiplatelet agent	32 (84)	27 (79)	.60[b]
Anticoagulant agent	6 (16)	8 (24)	.41[b]
Statin	32 (84)	31 (91)	.37[b]
Antihypertensive agent	34 (89)	31 (91)	.81[b]
Treated leg, right	19 (50)	22 (65)	.21[b]
Disease location			.81[b]
Suprainguinal	6 (16)	4 (12)	
Infrainguinal	23 (61)	23 (68)	
Supra- and infrainguinal	9 (24)	7 (21)	
Previous revascularization in treated leg	16 (42)	12 (35)	.55[b]
PWD, m	109 ± 70	111 ± 77	.90[a]
MWD, m	267 ± 177	263 ± 170	.94[a]
Resting ABI	0.53 ± 0.16	0.56 ± 0.15	.33[a]
Postexercise ABI	0.35 ± 0.17	0.33 ± 0.13	.52[a]
Vascuqol-6	13.4 ± 3.0	13.7 ± 3.3	.68[a]
EQ-5D-5L index	0.65 ± 0.20	0.70 ± 0.12	.20[a]

ABI, Ankle-brachial index; *MWD*, maximal walking distance; *PWD*, pain-free walking distance.
Data presented as mean ± standard deviation for continuous variables and as number (%) for categorical variables.
[a]Independent samples *t*-test.
[b]χ^2 Test.

(clinically classified as Fontaine IIb, corresponding to Rutherford class 2-3), both PWD and MWD increased in the treatment group compared with the sham control group.

To the best of our knowledge, the present study is one of the first double-blind randomized controlled trials to show that INP treatment increases the walking distance for patients with IC. Our findings are in line with the results reported by a placebo controlled study from Denmark, which also described an effect on walking distances.[18] However, only the within-group changes had been reported in that study.[18] Older studies have reported similar findings for patients with PAD,[13,16,17,31] but these were mainly case reports and patient series. However, two recent placebo controlled trials concluded that INP treatment does not provide additional effects to SET or home-based physical activity and lifestyle changes in increasing the walking capacity in patients with IC.[19,20] One explanation might be that the effect of increased physical activity surpasses the potential effects of INP treatment. Another explanation might be that the patients were treated with INP for only 30 and 40 minutes two and three times each week for 6 weeks, respectively, because that INP system required in-hospital treatment instead of at-home treatment. Hence, the frequency and length of the INP treatments were significantly lower than those used in the present study. Because INP applied to the lower leg induces acute

Table II. Analysis of covariance for all patients (N = 63)

Variable	No. available for analysis	Estimated treatment effect (95% CI)	*P* value
PWD, m	63	50 (11-89)	.014
MWD, m	63	42 (−14 to 97)	.14
Resting ABI	60	0.01 (−0.04 to 0.07)	.65
Postexercise ABI	47	0.04 (−0.02 to 0.10)	.19
Blood flow, mL/100 mL tissue			
Resting	59	0.5 (−0.6 to 1.6)	.34
Postischemic	53	0.4 (−1.1 to 2.0)	.58
Vascuqol-6	63	−0.11 (−1.53 to 1.31)	.89
EQ-5D-5L index	57	0.01 (−0.05 to 0.08)	.67
EQ-5D-5L VAS	63	5.3 (−4.7 to 15.4)	.29

ABI, Ankle-brachial index; *CI*, confidence interval; *MWD*, maximal walking distance; *PWD*, pain-free walking distance; *VAS*, visual analog scale.

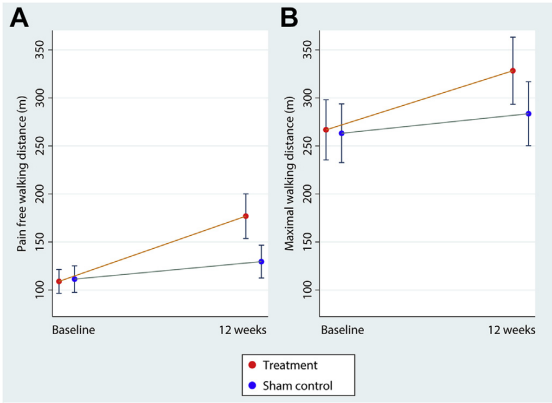

Fig 3. Pain-free walking distance (PWD) **(A)** and maximal walking distance (MWD) **(B)** at baseline and after 12 weeks of treatment. *Dots* indicate mean values; *error bars*, standard errors.

rhythmical fluctuations in blood flow,[22,32,33] which might promote long-term favorable effects leading to an increased walking capacity, it is reasonable to assume that a higher treatment frequency would be favorable and might also be necessary to achieve clinical effects.

In patients with a baseline PWD of <200 m, we observed an increase in both PWD and MWD in the treatment group compared with the sham control group. Although determined from a subgroup analysis, this finding indicates that the patients with the most symptomatic disease might benefit the most from INP treatment. Multiple studies have documented the beneficial effects of SET programs on walking capacity, functional status, and quality of life in patients with IC.[5,34-38] However, a systematic review from 2016 concluded that only one third of patients with IC were suitable for or willing to undertake SET.[9] Hence, the current guidelines recommending SET might not be applicable to most patients with IC. Although SET should be the first choice of treatment for patients with IC, INP treatment might be a useful supplement when SET is unavailable or for patients unable or unwilling to participate in SET.

The between-group changes in PWD in the present study were lower than assumed in the power calculations. However, to the best of our knowledge, no consensus has been reached regarding the minimal clinically important difference in walking performance after interventions in patients with IC. It is probably dependent on the disease severity, comorbidities, and the patient's subjective judgment. Pharmacologic agents such as cilostazol and pentoxifylline have market approval in Europe and the United States, with an indication of improving leg symptoms in patients with IC. A Cochrane review from 2014 estimated that cilostazol could increase the PWD by 31 m (95% CI, 22-40) and MWD by 43 m (95% CI, 18-68) compared with placebo.[39] Another Cochrane review from 2015 reported an improvement in PWD of −33.8% to 73.9% and in MWD of 1.2% to 156% with pentoxifylline. However, statistical tests were not performed because of insufficient data.[40] In the present study, we found an estimated treatment effect on the PWD of 50 m and a treatment effect for patients with a PWD <200 m of 42 m for PWD and 62 m for MWD. Thus, INP treatment is competitive to drug treatment in increasing the walking capacity of patients with IC.

Treatment of the calf or foot using intermittent pneumatic compression (IPC) has also been suggested to improve the walking distance for patients with IC.[11] Both INP and IPC increase arterial blood flow acutely when applied to the lower limb,[22,33,41] which might increase arterial shear stress, thereby inducing flow-mediated vasodilatation.[22,42] However, IPC is applied over a smaller area on the calf or foot and might not have the same microvascular effects on the whole lower leg compared with INP.

Endovascular or open surgery can be considered for patients with IC who have severely disabling symptoms and do not respond to SET.[7,43,44] A Cochrane review from

Table III. Analysis of covariance for baseline pain-free walking distance <200 m subgroup (N = 56)

Variable	No. available for analysis	Estimated treatment effect (95% CI)	*P* value
PWD, m	56	42 (2-83)	.042
MWD, m	56	62 (5-118)	.032
Resting ABI	53	0.00 (−0.06 to 0.06)	.91
Postexercise ABI	44	0.04 (−0.03 to 0.10)	.27
Blood flow, mL/100 mL tissue			
Resting	53	0.7 (−0.4 to 1.8)	.20
Postischemic	47	0.3 (−1.4 to 2.0)	.74
Vascuqol-6	56	−0.25 (−1.80 to 1.29)	.75
EQ-5D-5L index	53	0.02 (−0.05 to 0.08)	.61
EQ-5D-5L VAS	55	4.5 (−6.4 to 15.5)	.41

ABI, Ankle-brachial index; *CI*, confidence interval; *MWD*, maximal walking distance; *PWD*, pain-free walking distance; *VAS*, visual analog scale.

2018, which compared endovascular revascularization and conservative treatment of IC, reported a moderate effect on MWD and a large effect on PWD after 6 to 12 months.[45] However, no clear differences were shown between the groups after long-term follow-up.[45] Different outcome measures and study designs did not allow for direct comparisons to the findings in the present study. However, the effect of INP treatment after 3 months should be subject to further research.

In the present study, we did not observe any differences across the groups in the resting or postexercise ABI after 12 weeks of treatment. This is in line with the findings from a systematic review on the effects of exercise on IC, reporting an increased walking capacity without finding significant changes in the ABI.[5] Although we found an effect of INP treatment on walking distance, we did not find any differences in the quality of life parameters across the groups after 12 weeks of treatment. This does not correspond with the findings from studies investigating the effects of SET in IC.[36,38] One explanation might be that the improvement in the quality of life after participation in SET is also related to other factors than just the improvement in walking distance, such as increased physical activity and social interactions, which might not be obtained using INP treatment alone. Another explanation might be that the present study was underpowered to detect changes in quality of life across the groups.

The treatment group received −40 mm Hg INP, which is the standard INP level provided by the Conformitè Europëenne–marked FlowOx system commercially available in Europe. This INP level seemed to be well tolerated, and it is possible that a higher level could have been used. However, in a previous study from our research group, we did not observe any significant difference in the acute increase in arterial or skin blood flow at −60 mm Hg INP compared with −40 mm Hg INP.[22] Whether subgroups of patients could benefit from a higher INP level requires further investigation.

The prevalence of diabetes was higher in the treatment group than in the sham control group. Patients with diabetes are more prone to microangiopathy. Hence, the clinical effects observed in the present study could have resulted from positive effects on the arterial circulation or microcirculation, or both.

One patient in each group died of cardiac arrest during the intervention period. No clinical evidence was found to support a causal relationship between these events and the use of the treatment device or participation in the present study. The number of deaths in the present study did not allow for further statistical interpretations but underscores the high mortality for patients with symptomatic PAD.[46]

The present study had some limitations. We used −10 mm Hg INP in the sham device to make it appear identical to the active device without affecting the arterial blood flow. The similarity in compliance between the groups and the low withdrawal rate in the sham control group indicates that the patients really were unaware of their treatment allocation. However, a small effect might have resulted from the repetitive exposure to −10 mm Hg INP, leading to an underestimation of the treatment effect. More patients were lost to follow-up in the treatment group than in the sham control group because of user- or device-related problems. The use of the device requires some technical, cognitive, and motor capacity. In addition, some patients with a very small circumference of the lower leg might experience difficulty in achieving airtightness of the pressure chamber. Hence, the difference in those lost to follow-up between the two groups might have been random. Measurements of PWD is based on a subjective report of the onset of pain by the patient during the treadmill test. Thus, the results might have been affected by the fact that individuals might perform differently when they are being observed. However, in a double-blind, randomized controlled trial, the risk of bias from this phenomenon seems low. The patients were recruited from

the vascular surgery departments at three hospitals in Norway. Thus, one should be careful about generalizing the results to other patient populations or settings. However, whether the results from the present study are also applicable to patients with more severe stages of PAD should be the subject of further research.

CONCLUSIONS

The results from the present study have shown that treatment with −40 mm Hg INP for 1 hour in the morning and 1 hour in the evening for 12 weeks increased the PWD compared with sham treatment in patients with IC. For patients with a baseline PWD of <200 m, treatment with −40 mm Hg INP increased both PWD and MWD compared with sham treatment.

AUTHOR CONTRIBUTIONS

Conception and design: HH, IM, JH
Analysis and interpretation: HH, EP, LH, JH
Data collection: HH, EP, JH
Writing the article: HH
Critical revision of the article: HH, EP, LH, IM, AS, JH
Final approval of the article: HH, EP, LH, IM, AS, JH
Statistical analysis: HH, LH
Obtained funding: Not applicable
Overall responsibility: HH

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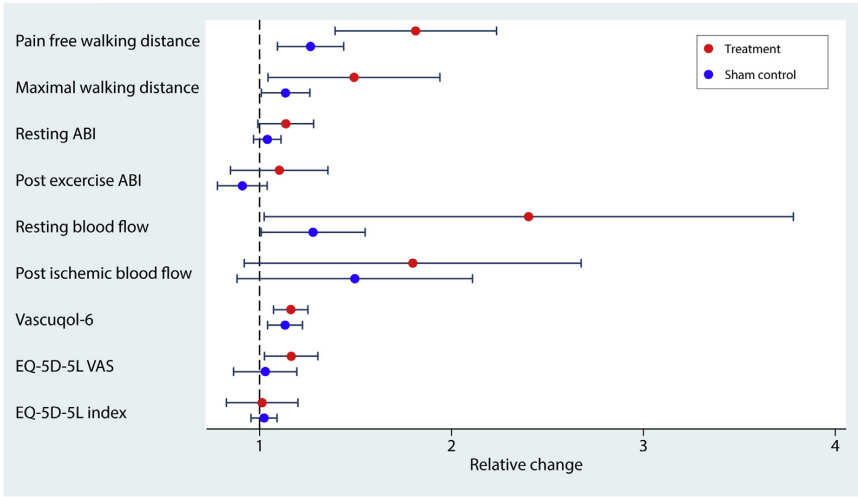

Supplementary Fig (online only). Relative within-group changes for all outcome variables. *Dots* indicate mean values; *error bars,* 95% confidence intervals. *ABI,* Ankle-brachial index.

Paper III

The effect of 12-week treatment with intermittent negative pressure on blood flow velocity and flowmotion, measured with a novel Doppler device (earlybird). Secondary outcomes from a randomized sham-controlled trial in patients with peripheral arterial disease.

Short title: INP effects on blood flow, measured with earlybird.

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Pettersen, Erik Mulder: none
Hoel, Henrik: employed by Otivio AS with funding from The Research Council of Norway (grant 285758).
Torp, Hans: inventor and shareholder of CIMON Medical AS
Hisdal, Jonny: none
Seternes, Arne: none

Otivio AS and CIMON Medical AS was not involved in the study design; collection, analysis, or interpretation of data; manuscript writing; or the decision to submit the manuscript for publication.

Abbreviations:
INP, intermittent negative pressure; PAD, peripheral arterial disease; v_{mean}, mean blood flow velocity; PWD, pain-free walking distance; MWD, maximal walking distance

Abstract

Objectives

Treatment with intermittent negative pressure (INP) is proposed as an adjunct to standard care in patients with peripheral arterial disease (PAD). The aims of this study were to evaluate the applicability of a novel ultrasound Doppler device (earlybird) to assess blood flow characteristics in patients with PAD during a treatment session with INP, and whether certain flow-properties could determine whom could benefit INP treatment.

Methods

Secondary outcomes of data from a randomized sham-controlled trial were explored. Patients were randomized to 12 weeks of treatment with 40 mmHg or 10 mmHg INP, for one hour twice daily. Earlybird blood flow velocity recordings were made before and after the 12-week treatment-period and consists of a 5-minute recording in rest, 3-minute during INP treatment and 5-minute recording after ended INP test-treatment. Mean blood flow velocity (v_{mean}), relative changes in flow and frequency spectrum by Fourier-transform of the respective bandwidths of endothelial, sympathetic, and myogenic functions, were analyzed for the different series of blood flow measurements.

Results

In total, 62 patients were eligible for analysis, where 32 patients were treated with 40 mmHg INP. The acquired recordings were of good quality and was used for descriptive analyses of flow characteristics. An immediate increase in v_{mean} during the negative pressure periods of the INP test-treatment was observed in the 40 mmHg INP treatment group at both pre- and post-test. There was a significant difference between the treatment groups, with a difference between the medians of 13.7 ($p < 0.001$) at pretest and 10.7 ($p < 0.001$) at posttest. This

finding was confirmed with spectrum analysis by Fourier-transform of the bandwidth corresponding to INP treatment. The change in amplitude corresponding to myogenic function after 12 weeks of treatment, was significantly different in favor of the 40 mmHg INP treatment group. We were not able to detect specific flow characteristics indicating whom would benefit INP-treatment.

Conclusions

Earlybird is an applicable tool for assessing blood flow velocity in patients with PAD. Analysis of the flow velocity recordings shows that INP induce an immediate increase in blood flow velocities during INP. The positive effects of INP may be attributed to recruitment of arterioles, and thereby increasing blood flow. In these analyses no flow characteristics was determined which could predict whom would benefit INP-treatment.

Keywords

Flowmotion, flow mediated dilation, ultrasound, Doppler, innovation, intermittent negative pressure,

Introduction

Standard of care for patients with peripheral arterial disease (PAD) includes optimizing lifestyle risk factors and medical treatment, as well as exercise treatment, preferably supervised exercise treatment [1]. A proportion of the patients are not suitable for exercise treatment [2-4], and a wide range of non-invasive non-pharmaceutical treatment options for PAD have emerged, such as intermittent pneumatic compression and electronic nerve and muscle stimulators [5]. Intermittent negative pressure (INP) applied to the extremities was already introduced in the early 20th century as a non-invasive method to improve peripheral blood circulation [6]. Several studies have suggested additional effect of INP-treatment to the standard care for patients with PAD on walking capacity [7, 8], although two recent randomized controlled studies found no additional beneficial effect [9, 10].

Our study group has previously shown that INP causes an acute increase in arterial blood flow [6, 11, 12]. The mechanism-of-action is proposed to be an increase in the arteriovenous pressure gradient promoting arterial flow [6]. Opposed to constant negative pressure, INP avoids vasoconstriction of the arterioles caused by the venoarteriolar reflex. Holder et al. [13] proposed that fluctuations in shear pattern, induced by INP-treatment, promotes endothelial function. The most effective INP-treatment protocol to induce an acute increase in arterial flow was found to be ten seconds of 40 mmHg negative pressure and seven seconds of atmospheric pressure [11, 12, 14]. With this treatment protocol, hard-to-heal wounds showed a tendency to heal after INP-treatment [15, 16].

We have earlier published results from a randomized sham-controlled trial, including 63 patients with intermittent claudication randomized to 12 weeks of 40 mmHg INP-treatment or sham treatment with 10 mmHg INP for one hour, twice daily, in a home-based setting [17]. The main finding was that INP-treatment increased pain-free walking distance (PWD) in the 40 mmHg INP treatment-groups, with an estimated treatment effect of 50

meters (95 % CI 11, 89), p = 0.014, compared to the 10 mmHg INP treatment group [17]. In a follow up study of ten patients who were treated for 24 weeks, with INP, a mean increase in PWD and maximal walking distance (MWD) was found [18]. However, in both studies a proportion of the patients did not show an improvement in walking capacity. It may therefore be clinically relevant to find criteria that could select whom would benefit from INP-treatment.

Earlybird is novel ultrasound Doppler monitoring device, with a single element highly sensitive transducer [19, 20]. The large aperture area of 80 mm^2, makes it easy to place over the vessels of interest. Earlybird correlates well with laser Doppler flowmetry and pulsed wave Doppler to assess microcirculatory function in healthy subjects [20] and it measures volume flow rate in arteriovenous fistulas for hemodialysis with comparable accuracy as duplex ultrasound [21]. The underlying technology has been used to monitor cerebral blood flow velocities in neonates [22, 23].

The aim of this study was to evaluate the applicability of a novel Doppler ultrasound device (earlybird), to assess blood flow characteristics in patients with PAD during a treatment session with INP and whether certain flow-properties could determine whom could benefit INP treatment.

Materials and methods

This is an exploratory study of secondary outcomes from an earlier published randomized sham-controlled multicenter trial on the effect of 12-weeks treatment with INP on walking distance in patients with intermittent claudication [17].

2.1 Patients

Between January and September 2019, patients were enrolled from three vascular surgery departments in Norway (St. Olavs Hospital, Trondheim, Oslo University Hospital,

Oslo, and Sørlandet Hospital, Kristiansand). Data collection was completed in 2019. A total of 72 patients were included and randomized, in a 1:1 ratio, to either a 10 mmHg or 40 mmHg INP treatment. Of these, 63 patients completed the 12-week intervention. One patient was excluded due to software failure during earlybird recordings, as described in the Consort Flow Diagram, figure 1. Labeling of the treatment devices was performed by the producer (Otivio AS) by a person not involved in patient recruitment or data collection. Patients and participating personnel were blinded to the group allocation.

2.2 Intermittent negative pressure

INP was applied to the treated leg using a custom-built boot with a proximal sealing zone, figure 2. A hose connected the boot to a control unit (FlowOx 2.0, Otivio AS, Oslo, Norway), which provided periods of alternating seven seconds of atmospheric and ten seconds of 10 mmHg or 40 mmHg negative pressure. After randomization the patients were given and trained in their personal device. The patients' most limiting leg was decided based on a treadmill test. They were instructed to treat that leg for one hour in the morning and one hour in the evening at home for 12 weeks.

2.3 Treadmill test

PWD and MWD were measured on a treadmill using a ramp protocol at a constant speed of 3.2 km/h starting at 0 % slope and increasing by 2 % every two minutes [24].

2.4 Blood flow measurement

Blood flow velocity measurements were recorded with earlybird, a novel unfocused ultrasound Doppler device, which we have validated in an earlier study [20]. Earlybird consist of a highly sensitive single element Doppler transducer [19], scanner and inhouse produced interface (MATLAB, MathWorks ® R2018a), figure 2.

The ultrasound Doppler probe was attached to the posterior tibial or dorsal pedal artery at the treated leg. The Doppler probe was mounted to provide an angle to the skin of approximately 60°, however the exact insonation angle related to the vessel of interest is not known. All equipment was attached before the leg was lowered into the INP-boot. The patients were seated comfortable and asked to relax without moving. They were left undisturbed while the measurements were recorded.

Earlybird measurements were done both before (pretest) and after the 12-week intervention period (posttest). We recorded a 5-minute earlybird-recording at rest (baseline). After a short break we performed a 9-minute recording consisting of 1-minute recording before INP test-treatment was started (pre INP-period), a 3-minute recording during INP-treatment sequence (INP-period), and a 5-minute recording after the INP-period was stopped (post INP-period), figure 3. All recordings were saved for post processing.

2.5 Data analysis and statistics

All recorded Doppler flow curves were optimized regarding sample volume, gain and signal tracing to improve signal quality. The Doppler traces were analyzed in MATLAB and exported to excel with mean blood flow velocities (v_{mean}) for each of the predefined recorded periods for both pre- and post-test and includes baseline, pre INP-periods, INP-period, and post INP-period. Within the INP-period, the Doppler velocity curves of the negative and atmospheric pressure-periods were analyzed separately. Although measurement for peak systolic, mean and end-diastolic flow velocity for all relevant time periods were acquired, only v_{mean}, were used for further analyses. Flow velocities are reported in arbitrary units (AU) due to unknown exact insonation angle to the vessel measured, figure 3.

Power spectrum analysis by Fourier-transform was applied to the Doppler blood flow velocity-curves, presenting frequency specter in a logarithmic scale. Spectrum analyses were performed for baseline, INP-period, and post INP-period. We calculated the area under the

power spectrum curve of each frequency band corresponding to specific flowmotion characteristics; 0.007 to 0.02 Hz , 0.02 to 0.06 Hz, 0.06 to 0.2 Hz, 0.2 to 0.6 Hz and 0.6 to 1.8 Hz associated to endothelial, sympathetic, vascular smooth muscle, respiratory and heart activity, respectively [25-27], figure 4. Amplitude was normalized to v_{mean}.

Descriptive statistics is presented as median, with its 25th and 75th percentile, or mean, with its 95 % confidence intervals for continuous variables, and the number with its percentage for categorical variables. Normality was assessed with Shapiro-Wilk test. One-Sample Wilcoxon Signed Rank for differences within the groups, Mann-Whitney U test for difference between the groups and Kruskal-Wallis H for difference between categories, were used. For post hoc analysis Mann-Whitney U for pairwise comparison with Holm-Bonferroni correction was used. Spearman's rank correlation (r_s) was used for correlation analysis. Statistical analyses were done in SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

2.6 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (reference no 2018/748) and registered in Clinical trials.gov (NCT03640676). The use of earlybird was approved by the Norwegian Government of Health, June 12th, 2017. All study participants gave their written informed consent before inclusion.

2.7 Funding

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Results

In total 62 patients were eligible for analysis, of these 30 were randomized to 10 mmHg and 32 to 40 mmHg INP-treatment. One posttest baseline-measurement in the 10 mmHg INP treatment-group, and one complete posttest measurement for one patient treated with 40 mmHg INP, were not recorded and not available for analyses. There were no demographic differences between the groups, except a larger proportion of patients with diabetes in the 40 mmHg INP treatment group, table 1.

3.1 Flow velocity recordings and characteristics

The acquired earlybird-recordings were of good quality and eligible for further analyses of changes in v_{mean}, example of flow velocity curve is given in figure 3. Treatment with 40 mmHg INP, induce an immediate increase in v_{mean} for the negative pressure period during the 3-minute INP-period (19.4% (3.1, 41.2), $p < 0.001$ for pretest and 9.4% (2.2, 24.9), $p < 0.001$ for the posttest). There is a significant difference between the treatment groups, in favor of 40 mmHg INP, difference between medians 13.7 ($p < 0.001$), and 10.7 ($p < 0.001$), pre- and post-test respectively, table 2.

There is a significant relative increase in v_{mean}, from the pre- to the post INP-period in the 40 mmHg INP treatment group (median increase 10.5% (-0.7, 19.2), $p < 0.001$), but only at the pretest. For the posttest there was no significant relative increase (median increase 2.1% (-5.9, 19.9), $p = 0.124$). Neither was there a significant relative increase in v_{mean} from pre- to post INP-period for the 10 mmHg INP treatment-group ($p = 0.102$ and $p = 0.360$), nor a difference between the two treatment groups for pre- and post-test ($p = 0.260$ and $p = 0.492$, respectively), table 2.

3.2 Fourier-transformation and analysis of INP-response

INP-treatment increased the normalized amplitude of the power density of area under the curve for 1/17 Hz bandwidth, corresponding to the frequency of the INP treatment-cycle. Both at pre- and post-test there was a significant difference between the treatment groups, in favor of 40 mmHg INP-treatment (difference between medians 4.9, $p < 0.001$ and 2.3, $p < 0.001$, respectively), table 3 and figure 5. No change in normalized amplitude for 1/17 Hz bandwidth was seen at the posttest, compared to the pretest (median -0.47 (-2.4, 1.3), $p = 0.271$ and -0.6 (-3.7, 1.1), $p = 0.171$, for 10 mmHg and 40 mmHg INP treatment groups respectively), table 4 and figure 5.

3.3 Fourier-transformation and analysis of flowmotion

We analyzed the Fourier-transformation for the 5-minute baseline recordings of the respective bandwidths for endothelial, sympathetic, and myogenic function (0.007 – 0.02, 0.02 – 0.06 and 0.06 – 0.2 Hz respectively), figure 4.

There was a difference between the 10 and 40 mmHg INP treatment-groups for the change, from pre- to posttest, in the normalized amplitude corresponding to myogenic function (0.06 – 0.2 Hz), with a difference between the medians of 0.86, $p = 0.002$. At pretest, the 10 mmHg INP treatment-group presented a higher normalized amplitude corresponding to myogenic function (6.9 (4.7, 9.5)) compared to the 40 mmHg INP treatment group (4.9 (2.8, 7.7)), difference between the medians of 2.0, $p = 0.015$, table 3. Within the 10 mmHg INP treatment-group, a reduction in normalized amplitude corresponding to myogenic function was seen from the pre- to the post-test, median change -0.99 (-4.9, 0.01), $p = 0.003$. No change was seen within the 40 mmHg INP treatment-group at posttest, median change 0.85 (-1.3, 2.6), $p = 0.167$, table 4 and figure 6.

No difference was found for the normalized amplitude of the respective flowmotion bandwidths and categories for PWD (<50, 50-100, 100-150, and >150 meters) or for MWD (<100, 100-200, 200-300 and >300 meters), except for endothelial function categorized for

PWD at pretest (p = 0.041) and sympathetic function for the compiled amplitude for both treatment groups at the posttest (p = 0.046), supplementary material I. However, post hoc analyses showed no significant difference between the identified categories (p = 0.096 and 0.342 respectively). Between the 10 and 40 mmHg INP treatment-groups for the respective flowmotion bandwidths, no significant change in normalized amplitude from pre- to posttest was found, except for myogenic function as mentioned above. No differences between the categories for improvement in PWD or MWD, was found, supplementary material II.

3.4 Flow characteristics at pretest to predict outcome after 12 weeks of 40 mmHg INP-treatment.

In the 40 mmHg INP treatment-group we found no correlation between v_{mean} at the pre INP-period, relative increase in v_{mean} during INP-period or post INP-period at pretest, and improvement in PWD or MWD after 12 weeks of INP-treatment, table 5. There was no significant difference in the relative increase in v_{mean} for the different categories in improvement in PWD or MWD, figure 7. Neither was there a correlation between improved walking distance and normalized amplitude for flowmotion bandwidths, nor for the normalized amplitude for bandwidth corresponding to INP-treatment table 5 and supplementary material II.

Discussion

The main finding in this study of secondary outcomes of a randomized sham-controlled trial, was that earlybird is a feasible tool to measure blood flow velocity during ongoing treatment with INP in patients with PAD. Treatment with 40 mmHg INP elicits an immediate increase in blood flow velocity. This finding is confirmed in the spectrum analyses by Fourier-transformation of the normalized amplitude of the bandwidth corresponding to the INP-response (1/17 Hz), and are in line with recent studies on INP and the effects on micro-

and macro-circulation [11, 12, 14]. Evaluation of flowmotion at the post-test, showed a difference between the treatment groups of the change in normalized amplitude corresponding to myogenic function (0.06 – 0.2 Hz), in favor of 40 mmHg INP treatment-group. No differences were found for endothelial or sympathetic function.

Changes in amplitude of the bandwidth associated to myogenic function has been proposed to be caused by activity of the vasomotor cells in the arterioles [28]. The observed difference in change in myogenic function between the groups, after 12 weeks of INP-treatment, may indicate that the mechanically induced flowmotion pattern by INP has a positive impact on the myogenic regulation of arterioles, and thereby improve peripheral blood circulation and increase walking ability. Results from previous studies have demonstrated inhibited flowmotion patterns in patients with lower extremity atherosclerotic disease, especially endothelial activity [26, 27, 29, 30]. In the present study we did, however, not observe any association between the degree of reduced walking ability and the amplitude of the different flowmotion bandwidths, neither at the pre- nor post-test. The lack of findings may be due to limitations by the study.

Sundby [11] and Hoel [14], with colleagues, determined that INP-treatment of ten seconds of 40 mmHg negative pressure and seven seconds of atmospheric pressure was the optimal protocol to increase blood flow velocity. A constant negative pressure of 40 mmHg mimics the gravitational force in upright position [31], and leads to an increase in the arteriovenous pressure gradient and a temporary increase in blood flow [32]. Increased in-flow and reduced emptying of the venous reservoir increase the venous pressure and evens out the pressure gradient. The venoarteriolar reflex elicits vasoconstriction of arterioles which again reduce blood flow [32]. Oscillations with negative pressure, in contrast, extinguish the venoarteriolar reflex [6] and increase arterial and cutaneous blood flow in healthy individuals as well as in patients with peripheral arterial disease [11, 12]. Direct suction on the arteriolar

vascular bed or increased emptying of the venous reservoir may be two mechanisms of action. It is suggested that INP-treatment stimulates and promotes vascular health through mimicking blood flow fluctuations [6], and that this could be the etiology for the observed positive effect of INP-treatment. The fluctuations and increase in arterial flow increase shear stress, release vasoactive substances, and may lead to flow mediated dilation [13, 33, 34]. The main stimulus for improving vascular health may be the fluctuations in blood flow, more than increase in mean flow velocity [12, 13, 15, 35]. The difference between the treatment groups in change in myogenic function, found in this study, may contribute to further elucidate the mechanisms of effect of INP treatment, with the possible role of arterioles.

Within group analysis for 40 mmHg INP treatment-groups at pretest, showed a relative increase in n v_{mean} post INP-period when compared to pre INP-period. This was not present in the 10 mmHg INP-treatment-group, and no significant difference was seen between the groups. Neither was this confirmed in the posttest, nor by analysis of between groups difference. A relative increase in v_{mean} after a 3-minute INP test-treatment could be explained as flow mediated dilation [13]. Patients with a change in PWD ≤ 0 meters after 12 weeks of INP-treatment showed no increase in mean flow velocity after a 3-minute INP-period, figure 7. It would be intriguing to pursuit the thought that patients who do not produce flow mediated dilation after three minutes with 40 mmHg INP are more likely to not benefit from INP-treatment. Future, well powered, studies could clarify if certain flow characteristics could be used to select those who do not respond to INP-treatment.

Intermittent pneumatic compression (IPC) to the foot and calf, or thigh, in patients with PAD, has been demonstrated to increase walking distance and wound healing [5, 36-38]. IPC cause a post-compression hyperemia, seen as an increase in arterial blood flow and velocity measured by laser Doppler or ultrasound Doppler devices [36]. The mechanism is suggested to be a combination of stimulation of vasoactive substances, increase in the arterio-

venous pressure gradient, reduction in venous-arterial reflex, and stimulation of arterial collateralization [37, 38]. The mechanism of action of IPC are similar to those proposed for INP-treatment. The common feature being a manipulation of the venous reservoir, while INP may in addition perform an active suction on the capillary and arteriole vascular network.

In this study all blood flow velocity recordings were done with earlybird. Earlybird has a highly sensitive transducer and monitors blood flow in a large area, making it stable and easy-to-use. One of the greatest limitations with earlybird is that the insonation angle to the underlying vessel is not known. In this study it is therefore not possible to evaluate changes over time in absolute flow values, only relative change can be assessed. Information of change in absolute flow velocities may be lost. The recordings made were of good quality, making them eligible for further analysis. In other studies, examining the effects of INP-treatment, pulsed-wave Doppler and laser Doppler flowmetry have been used [12]. We find similar results as in these studies indicating that earlybird is a valid method for assessing and monitoring blood flow velocity.

4.1 Limitations

Flowmotion characteristics may vary between skin localizations as well as in time [26, 39]. The analysis of flowmotion, especially the low frequency specter of endothelial function (0.007 – 0.02 Hz), is complicated by the short recording-time in this study [39]. A 5-minute recording only capture one to two cycles of endothelial flowmotion, while more cycles are measured for higher frequencies. Longer recordings are therefore needed to fully assess flowmotion as response to INP treatment. This may be a potential explanation of the lack of association between flowmotion and walking distance.

The main results from the randomized trial showed only a modest clinical effect regarding PWD, while no significant effect for MWD [17]. This study was conducted without implementing a validated physiological test which could elicit a vasomotor response but used

a 3-minute INP test-treatment. The moderate effect of INP treatment, small sample size, and the fact that only 32 of the 62 study participants was tested with 40 mmHg INP test-treatment, due to the double blinded nature of the study, may mask flow characteristics that could determine whom would benefit INP-treatment.

Lack of association between flow characteristics, walking distance and effect of INP treatment may be due to large variation in measurements and low sample size.

Conclusion

The novel ultrasound Doppler device, earlybird, is an applicable tool for assessing blood flow velocity in patients during treatment with INP in patients with intermittent claudication. Analysis of the acquired flow velocity recordings shows that arterial blood flow velocities immediate increase during INP-treatment. There is a difference between the groups in favor of 40 mmHg INP, in vasomotor activity corresponding to an increase in myogenic function. This may indicate an involvement of vascular smooth muscle cells of the arterioles and may contribute to the understanding of the mechanism of action of INP treatment. I this study, we did not find specific flow characteristics that is able to determine whom would benefit INP-treatment. Additional well-designed studies examining the effect and selection criteria of INP-treatment are needed to fully understand the underlying mechanism of action and to reliably propose clinical decision criteria for indications for INP-treatment.

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Table 1: Baseline characteristics of patients eligible for analyses

Variable	40 mmHg INP n = 32	10 mmHg INP n = 30
Age (years)	72 (67, 77)	73 (69, 78)
Male sex	19 (59)	23 (72)
Body mass index (kg/m^2)	26.7 (24.7, 30.7)	27.1 (23.9, 30.6)
Ankle-brachial-index (ABI), at rest	0.50 (0.42, 0.67)	0.57 (0.47, 0.64)
Pain-free walking distance (PWD) (m)	92 (47, 150)	86 (50, 152)
Maximal walking distance (MWD) (m)	233 (139, 313)	250 (103, 379)
Smoking		
Current	11 (34 %)	10 (31 %)
Previous	18 (56 %)	17 (53%)
Never	3 (9 %)	3 (9 %)
Diabetes mellitus	16 (50 %)	6 (19 %)
Chronic renal failure	2 (6 %)	4 (13 %)
Hypertension	26 (81 %)	25 (78 %)
Hypercholesterolemia	18 (56 %)	25 (78 %)
Coronary artery disease	14 (44 %)	17 (53 %)
Cerebrovascular disease	3 (9 %)	8 (25 %)
Antiplatelet agent	27 (84 %)	24 (75 %)
Anticoagulation	5 (16 %)	6 (19 %)
Statin	26 (81 %)	28 (88 %)
Antihypertensiva	28 (88 %)	28 (88 %)

INP, intermittent negative pressure; continuous data presented as median with its 25th and 75th percentile

Table 2: Acute effects on flow velocity of INP-treatment

	10mmHg INP (n =30) Median* (25th, 75th percentile)	40 mmHg INP (n = 32) Median* (25th, 75th percentile)	Absolute difference between medians†
Pretest			
Pre INP-period (AU)	6.13 (3.86, 9.13)	6.52 (3.89, 9.52)	0.39, p = 1.000
Relative increase in v_{mean} during negative-pressure-periods in the INP-period, compared to pre INP-period	5.7 % (-6.8, 12.2) p = 0.106	19.4 % (3.7, 41.2) p < 0.001	13.7, p = 0.001
Relative increase in v_{mean} post INP-period, compared to pre INP-period.	4.9 % (-4.4, 15.7) p = 0.102	10.5 % (-0.7, 19.2) p < 0.001	5.6, p = 0.260
Posttest (after 12-week INP-treatment)			
Pre INP-period (AU)	7.62 (4.60, 11.86)	6.21 (4.02, 10.20)	1.41, p = 0.379
Relative increase in v_{mean} during negative-pressure-periods in the INP-period, compared to pre INP-period	-0.3 % (-5.4, 9.1) p = 0.355	9.4 % (2.2, 24.9) p < 0.001	10.7, p = 0.003
Relative increase in v_{mean} post INP-period, compared to pre INP-period.	3.0 % (-7.9, 12.9) p = 0.360	2.1 % (-5.9, 19.9) p = 0.124	-0.9, p=0.493

INP, intermittent negative pressure; AU, arbitrary units; v_{mean}, mean blood flow velocity;
*One-Sample Wilcoxon Signed Rank test; †Mann-Whitney U

Table 3: Normalized amplitude for flowmotion-bandwidths (Fourier-transform) at pre- and posttest.

	Pretest (before 12-week INP treatment)			Posttest (after 12-week INP treatment)		
	10 mmHg INP median (25th, 75th)	40 mmHg INP median (25th, 75th)	p between groups†	10 mmHg INP median (25th, 75th)	40 mmHg INP median (25th, 75th)	p between groups†
INP-treatment (1/17 Hz)						
	3.9 (2.7, 5.0)	8.8 (5.1, 10.5)	< 0.001	3.4 (2.3, 4.7)	5.7 (4.6, 10.2)	< 0.001
Flowmotion						
Endothelial function (0.007 – 0.02 Hz)						
	7.3 (4.4, 16.5)	7.7 (3.8, 15.9)	0.345	7.0 (5.1, 10.4)	7.5 (4.5, 13.3)	0.807
Sympathetic function (0.02 – 0.06 Hz)						
	7.8 (5.2, 12.3)	6.9 (3.4, 11.4)	0.115	7.4 (5.7, 19.4)	7.2 (5.7, 10.1)	0.801
Myogenic function (0.06 – 0.2 Hz)						
	6.9 (4.7, 9.5)	4.9 (2.8, 7.7)	0.015	5.6 (4.6, 7.0)	5.8 (4.0, 8.2)	0.668

INP, intermittent negative pressure; †Mann-Whitney U

Table 4: Absolute change in normalized amplitude for flowmotion-bandwidths at posttest (after 12 weeks of INP treatment), compared to pretest.

	Absolute change in flowmotion from pretest to posttest				
	10 mmHg INP median (25th, 75th)	p within groups*	40 mmHg INP median (25th, 75th)	p within groups*	p between groups†
INP-treatment (1/17 Hz)					
	-0.47 (-2.4, 1.3)	0.271	-0.6 (-3.7, 1.1)	0.171	0.690
Flowmotion					
Endothelial function (0.007 – 0.02 Hz)					
	-0.44 (-8.9, 1.7)	0.304	-0.18 (-6.2, 5.0)	0.624	0.569
Sympathetic function (0.02 – 0.06 Hz)					
	0.20 (-5.6, 10.4)	0.699	0.73 (-2.6, 3.0)	0.537	0.391
Myogenic function (0.06 – 0.2 Hz)					
	-0.99 (-4.0, 0.01)	0.003	0.85 (-1.3, 2.6)	0.167	0.002

INP, intermittent negative pressure; *(One-Sample) Wilcoxon Signed Rank test; †Mann-Whitney U

Table 5: Correlation between flow characteristics for v_{mean} at pretest, and improvement in pain-free (PWD) and maximal walking distance (MWD) for the 40 mmHg INP treatment group after 12 weeks of INP-treatment.

Change in walking distance from pre- to post-test for:		Blood flow velocity (v_{mean})			Fourier-transform			
		pre INP-period	Relative increase INP-period	Relative increase (post INP-teriod)	Endothelial function (normalized)	Sympathetic function (normalized)	Myogenic function (normalized)	Amplitude INP (1/17 Hz)
PWD	r_s	-0,118	-0,110	0,178	0,241	0,310	0,264	0,317
	p	0,521	0,550	0,329	0,184	0,084	0,144	0,077
MWD	r_s	-0,239	0,176	-0,134	0,176	0,171	0,235	0,007
	p	0,187	0,335	0,465	0,335	0,350	0,196	0,970

r_s, spearman's rank correlation coefficient; p, significance level (2-tailed); INP, intermittent negative pressure; Hz, hertz, v_{mean}, mean blood flow velocity; n = 32 for all groups

Figures

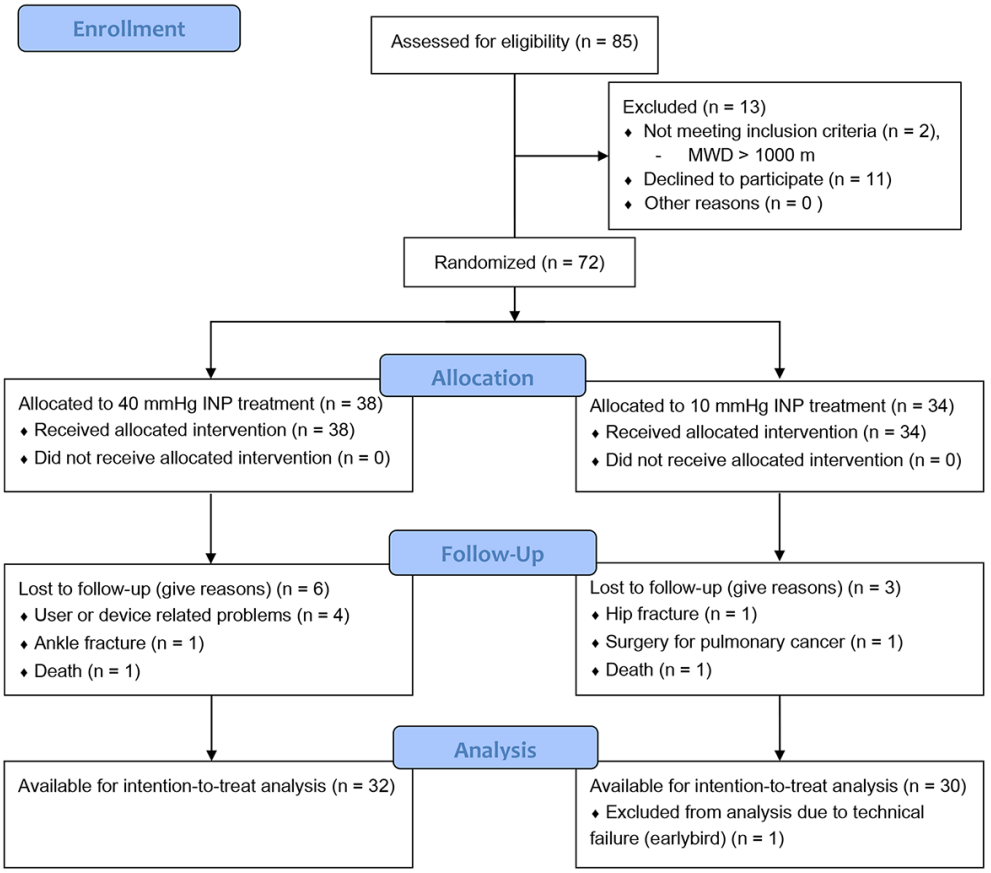


Figure 1: CONSORT (consolidated standards of reporting trials) flow diagram

The flow diagram is showing inclusion, exclusion, treatment allocation, and outcomes. INP, Intermittent negative pressure; MWD, maximal walking distance.

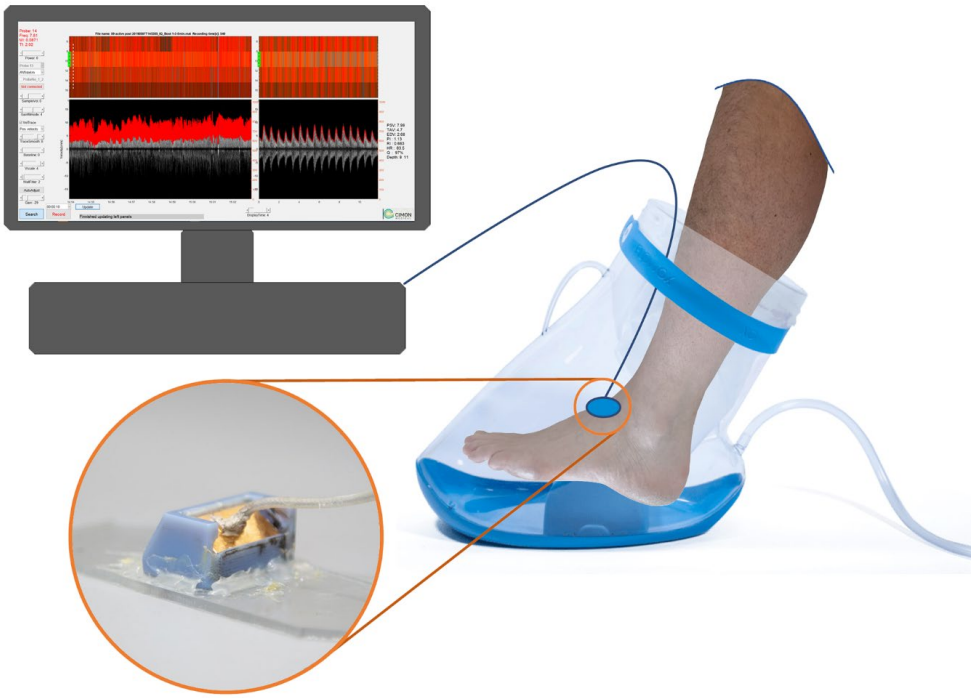


Figure 2: Illustration of the treated leg placed in an INP-treatment boot (FlowOx 2.0) There is a proximal sealing zone around the calf. The external pump is not visualized. The earlybird probe (highlighted picture) is connected to a scanner and monitor with the interface. (Illustration: Erik Mulder Pettersen/NTNU, photo: Karl Jørgen Marthinsen/NTNU)

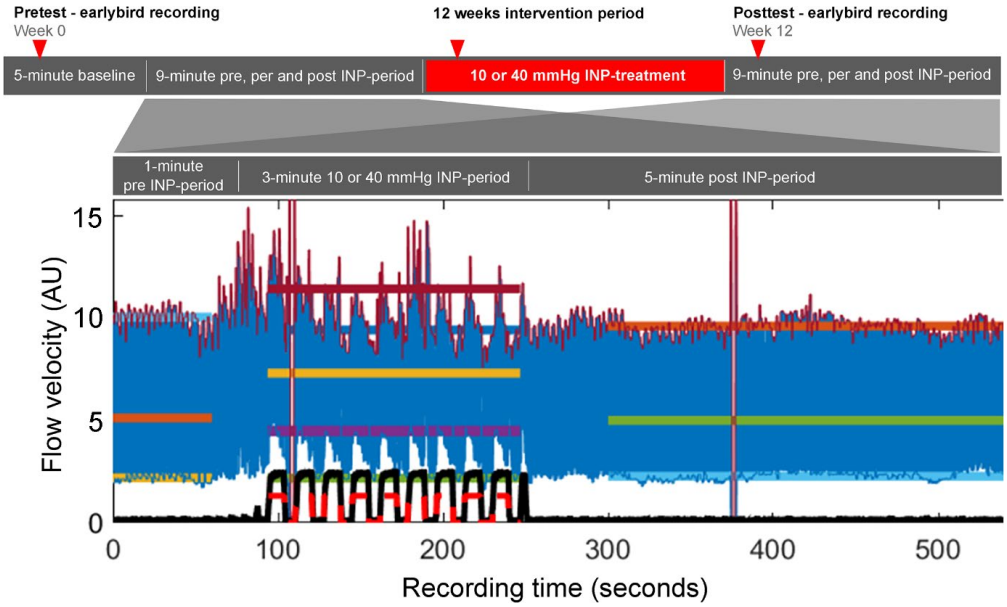


Figure 3: Overview of pre- and post-test earlybird recordings.

Mean blood flow velocity (v_{mean}) was calculated for baseline (not visualized), pre INP-period (orange), the negative-pressure periods of the INP-period (yellow), atmospheric-pressure periods of the INP-period (purple) and post INP-period (green) respectively. The black line indicates inverted negative-pressure periods.

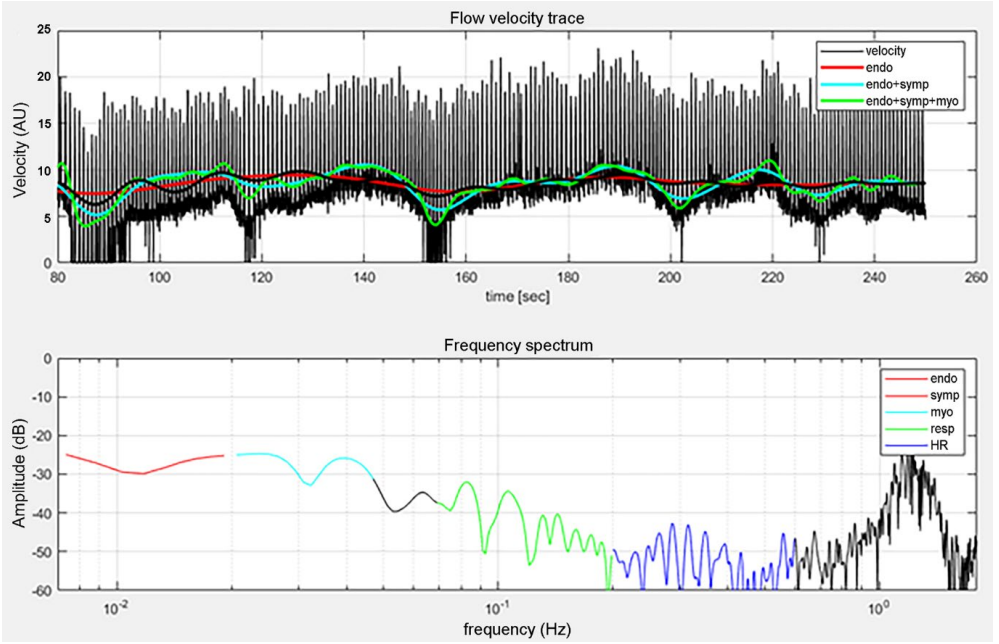


Figure 4: Example of frequency specter analysis for 5-minute baseline velocity trace. Bandwidths were set for endothelial (0.007 – 0.02 Hz), sympathetic (0.02 – 0.06 Hz), myogenic 0.06 – 0.2 Hz), respiratory (0.2 - 0.6 Hz) and heart rate (0.6 – 1.8 Hz) distribution frequency respectively.

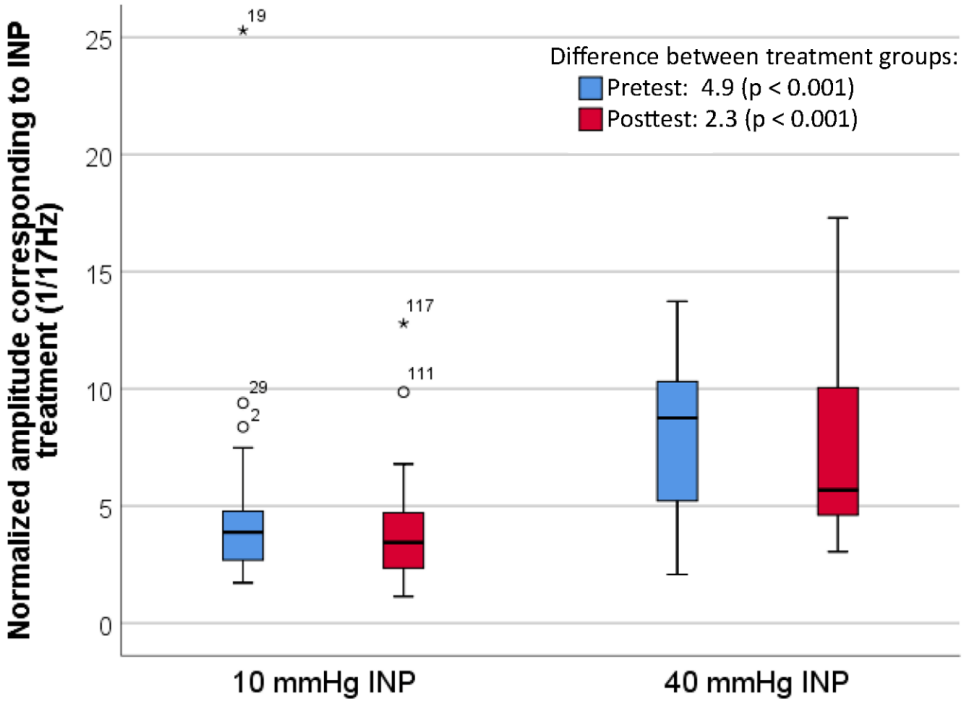
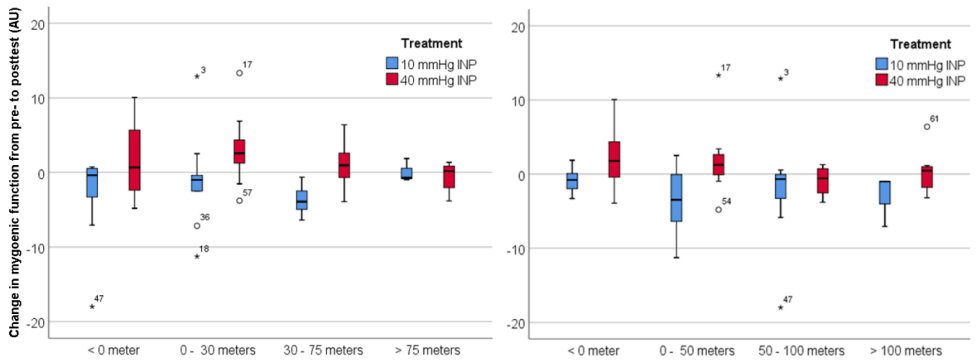


Figure 5: Normalized amplitude of the power density under the curve corresponding to INP treatment (1/17 Hz), for both treatment groups at pre- and posttest.

The absolute difference between the medians of the pre are posttest are presented, see also table 3. For change within the treatment groups at posttest, compared to pretest, see also table 4.



Improvement in PWD (m)	< 0	0–30	30–75	> 75	SUM
10 mmHg INP (n)	10	9	7*	3	29
40 mmHg INP (n)	4	9	8	10*	31
Total (n)	14	18	15	13	60

*missing samples n = 1 (total 2)

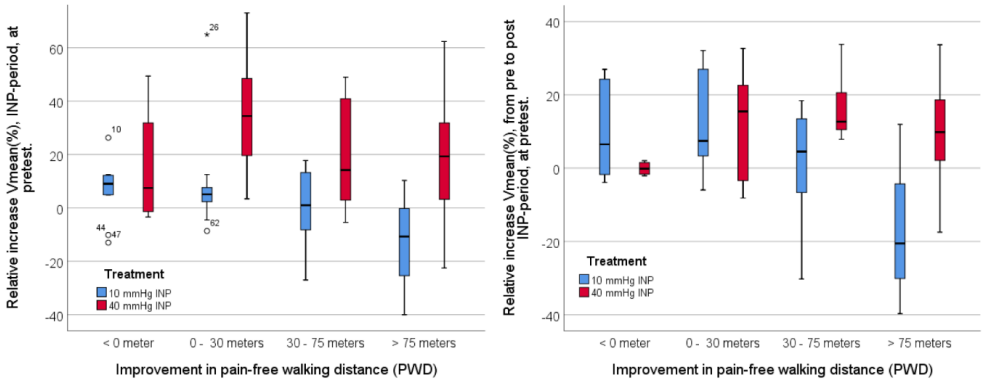
Improvement in MWD (m)	< 0	0–50	50–100	> 100	SUM
10 mmHg INP (n)	9	10	7*	3	29
40 mmHg INP (n)	10	9*	4	8	31
Total (n)	14	18	15	13	60

*missing samples n = 1 (total 2)

Figure 6: Absolute change in normalized amplitude for flowmotion-bandwidths for improvement in pain-free (PWD) and maximal walking distance (MWD) at posttest (after 12 weeks of INP-treatment), compared to pretest. Difference for the 10 and 40 mmHg INP-treatment-groups, between the categories of improvement in PWD: p = 0.234 and 40 mmHg INP: p = 0.182, respectively, and MWD: p = 0443 and 0.551, respectively. Difference between treatment groups: p = 0.002.

a)

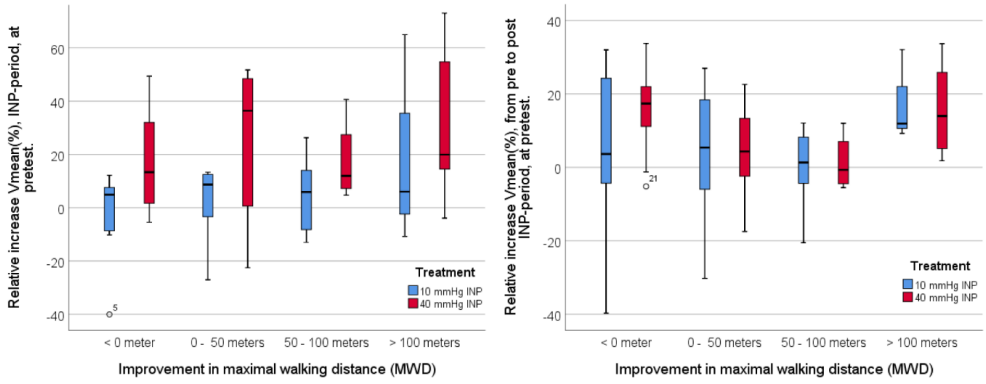
Improvement in PWD (m)	< 0	0 – 30	30 – 75	> 75	SUM
10 mmHg INP (n)	10	9	8	3	30
40 mmHg INP (n)	4	9	8	11	32
Total (n)	14	18	16	14	62



For improvement in PWD	10 mmhg	40 mmhg
relative increase in v_{mean} in INP-period	p = 0.467[a]	p = 0.362[a]
relative increase in v_{mean} from pre to post INP-period	p = 0.451[a]	p = 0.195[a]

b)

Improvement in MWD (m)	< 0	0 – 50	50 – 100	> 100	SUM
10 mmHg INP (n)	9	10	8	3	30
40 mmHg INP (n)	10	10	4	8	32
Total (n)	19	20	12	11	62



For improvement in MWD	10 mmhg	40 mmhg
relative increase in v_{mean} in INP-period	p = 0.730[a]	p = 0.521[a]
relative increase in v_{mean} from pre to post INP-period	P = 0.376[a]	p = 0.059[a]

Figure 7: a) Relative increase (%) in mean velocity (v_{mean}) at INP-period, and b) from pre to post INP-period, for categories in improved PWD and MWD. INP, intermittent negative pressure; PWD, pain-free walking distance; MWD, maximal walking distance, n, number. [a]Kruskal-Wallis H.

Supplementary material

Supplementary material I: Boxplots of normalized amplitude for flowmotion-bandwidths (endothelial, sympathetic, and myogenic function) for categories of pain-free (PWD) and maximal walking distance (MWD) at pretest and posttest (before and after 12 weeks of intermittent negative pressure (INP) treatment), for 10 and 40 mmHg INP treatment groups.

Normalized amplitude for flowmotion-bandwidths for pain-free walking distance (PWD) categories at pretest (before 12 weeks of INP-treatment).

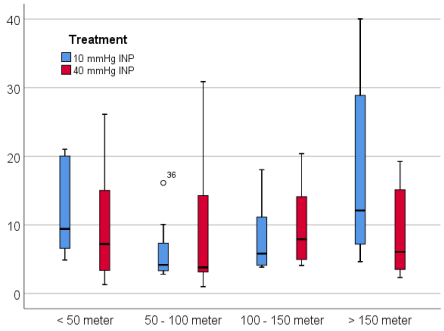
Pretest PWD (m)	< 50	50–100	100–150	>150	SUM
10 mmHg INP (n)	7	10	5	8	30
40 mmHg INP (n)	8	9	7	8	32
Total (n)	15	19	12	16	62

Normalized amplitude for flowmotion-bandwidths for pain-free walking distance (PWD) categories at posttest (after 12 weeks of INP-treatment).

Posttest PWD (m)	< 50	50–100	100–150	> 150	SUM
10 mmHg INP (n)	5	9*	7	8	29
40 mmHg INP (n)	4	5	10	12*	31
Total (n)	9	15	17	21	60

*missing samples n = 1 (total 2)

Endothelial function (0.007 - 0.02 Hz):



Pain-free walking distance (PWD) before 12 weeks of INP-treatment (pretest)

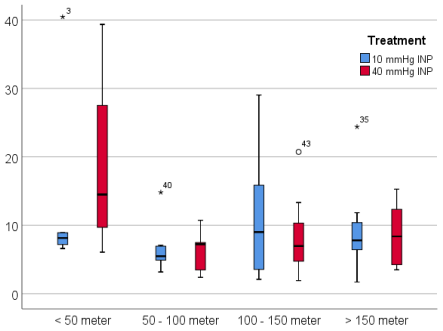
Difference between categories:

Total p = 0.124[a]

10 mmHg: p = 0.041[a]

40mmHg: p = 0.805[a]

Endothelial function (0.007 - 0.02 Hz):



Pain-free walking distance (meters) after 12 weeks of INP-treatment (posttest)

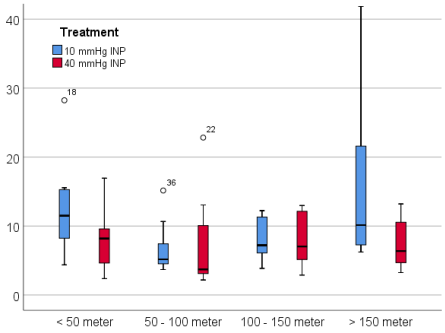
Difference between categories:

Total: p = 0.129[a]

10 mmHg: p = 0.320[a]

40mmHg: p = 0.212[a]

Sympathetic function (0.02 - 0.06 Hz):



Pain-free walking distance (PWD) before 12 weeks of INP-treatment (pretest)

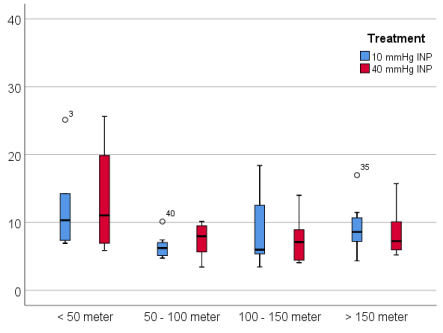
Difference between categories:

Total: p = 0.129[a]

10 mmHg: p = 0.070[a]

40mmHg: p = 0.734[a]

Sympathetic function (0.02 - 0.06 Hz):



Pain-free walking distance (meters) after 12 weeks of INP-treatment (posttest)

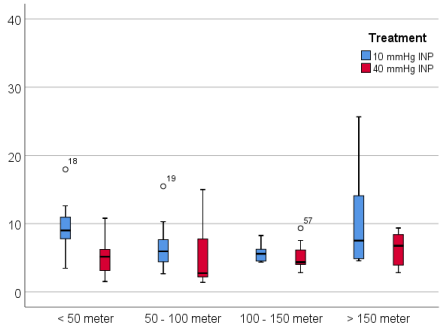
Difference between categories:

Total: p = 0.046[a]

10 mmHg: p = 0.131[a]

40mmHg: p = 0.421[a]

Myogenic function (0.06 - 0.2 Hz):



Pain-free walking distance (PWD) before 12 weeks of INP-treatment (pretest)

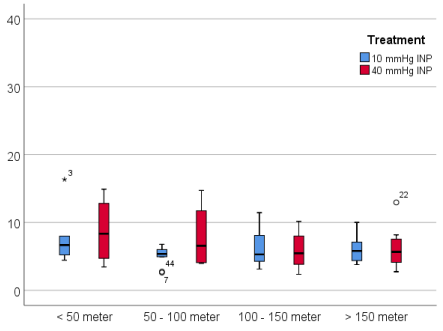
Difference between categories:

Total: p = 0.321[a]

10 mmHg: p = 0.215[a]

40mmHg: p = 0.578[a]

Myogenic function (0.06 - 0.2 Hz):



Pain-free walking distance (meters) after 12 weeks of INP-treatment (posttest)

Difference between categories:

Total: p = 0.558[a]

10 mmHg: p = 0.593[a]

40mmHg: p = 0.629[a]

[a]Kruskal-Wallis H

Normalized amplitude for flowmotion-bandwidths for maximal walking distance (MWD) categories at pretest (before 12 weeks of INP-treatment).

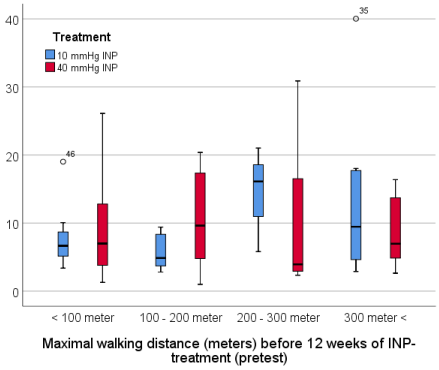
Pretest MWD (m)	<100	100 –200	200 –300	>300	SUM
10 mmHg INP (n)	7	7	3	13	30
40 mmHg INP (n)	5	8	10	9	32
Total (n)	12	15	13	22	62

Normalized amplitude for flowmotion-bandwidths for maximal walking distance (MWD) categories at posttest (after 12 weeks of INP-treatment).

Posttest MWD (m)	<100	100–200	200–300	>300	SUM
10 mmHg INP (n)	7	7	2*	13	29
40 mmHg INP (n)	4	6	6	15*	31
Total (n)	11	13	8	28	60

*missing samples n = 1 (total 2)

Endothelial function (0.007 - 0.02 Hz), for categories MWD:



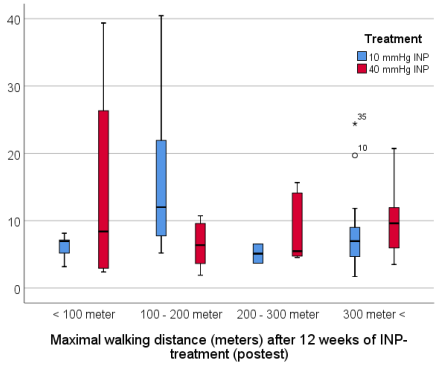
Difference between categories:

Total: p = 0.802[a]

10 mmHg: p = 0.401[a]

40mmHg: p = 0.763[a]

Endothelial function (0.007 - 0.02 Hz), for categories MWD:



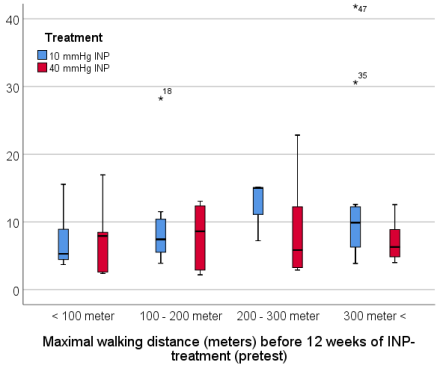
Difference between categories:

Total: p = 0.512[a]

10 mmHg: p = 0.125[a]

40mmHg: p = 0.696[a]

Sympathetic function (0.02 - 0.06 Hz), for categories MWD:



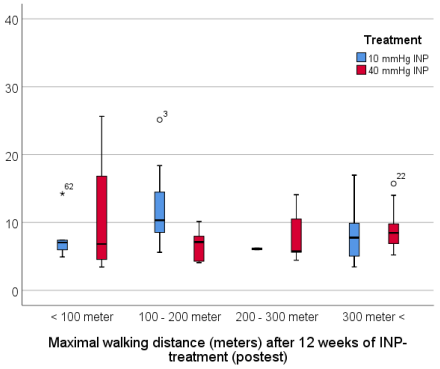
Difference between categories:

Total: p = 0.788[a]

10 mmHg: p = 0.291[a]

40mmHg: p = 0.988[a]

Sympathetic function (0.02 - 0.06 Hz), for categories MWD:



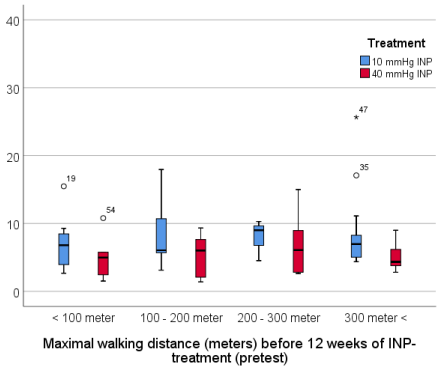
Difference between categories:

Total: p = 0.518[a]

10 mmHg: p = 0.239[a]

40mmHg: p = 0.538[a]

Myogenic function (0.06 - 0.2 Hz), for categories MWD:



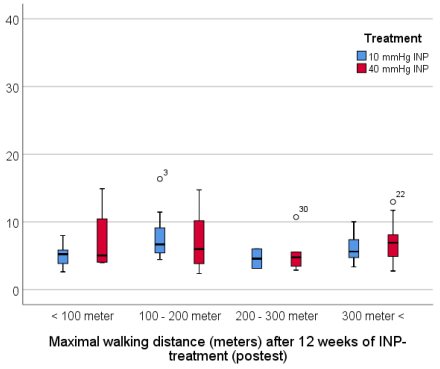
Difference between categories:

Total: p = 0.938[a]

10 mmHg: p = 0.871[a]

40mmHg: p = 0.841[a]

Myogenic function (0.06 - 0.2 Hz), for categories MWD):



Difference between categories:

Total: p = 0.245[a]

10 mmHg: p = 0.352[a]

40mmHg: p = 0.596[a]

[a]Kruskal-Wallis H

Supplementary material II: Boxplots of absolute change in normalized amplitude for flowmotion-bandwidths (endothelial, sympathetic, and myogenic function) for improvement in pain-free (PWD) or maximal walking distance (MWD) at posttest (after 12 weeks of intermittent negative pressure (INP) treatment), compared to pretest, for 10 and 40 mmHg INP treatment groups.

Absolute change in normalized amplitude for flowmotion-bandwidths for improvement in pain-free walking distance (PWD) at posttest (after 12 weeks of INP-treatment), compared to pretest.

Improvement in PWD (m)	< 0	0–30	30–75	> 75	SUM
10 mmHg INP (n)	10	9	7*	3	29
40 mmHg INP (n)	4	9	8	10*	31
Total (n)	14	18	15	13	60

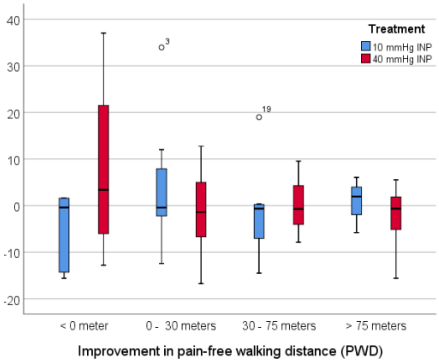
*missing samples n = 1 (total 2)

Absolute change in normalized amplitude for flowmotion-bandwidths for improvement in maximal walking distance (MWD) at posttest (after 12 weeks of INP-treatment), compared to pretest.

Improvement in MWD (m)	< 0	0–50	50–100	> 100	SUM
10 mmHg INP (n)	9	10	7*	3	29
40 mmHg INP (n)	10	9*	4	8	31
Total (n)	14	18	15	13	60

*missing samples n = 1 (total 2)

Change in endothelial function (0.007 - 0.02 Hz):

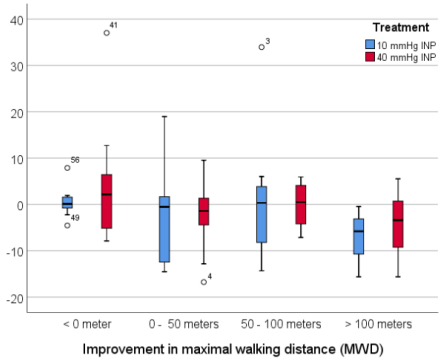


Difference between categories:

10 mmHg: p = 0.756[a]

40mmHg: p = 0.820[a]

Change in endothelial function (0.007 - 0.02 Hz):

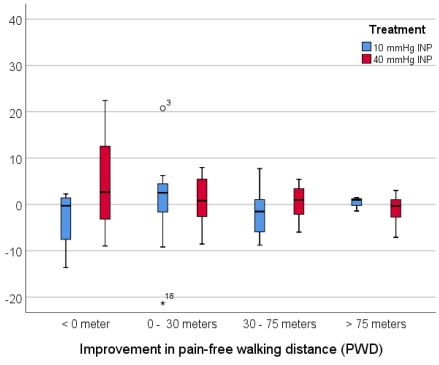


Difference between categories:

10 mmHg: p = 0.498[a]

40mmHg: p = 0.324[a]

Change in sympathetic function (0.02 - 0.06 Hz):

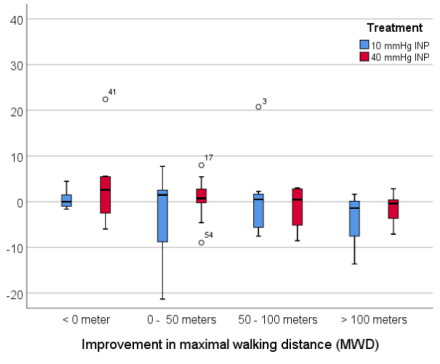


Difference between categories:

10mmHg: p = 0.437[a]

40mmHg: p = 0.580[a]

Change in sympathetic function (0.02 - 0.06 Hz):

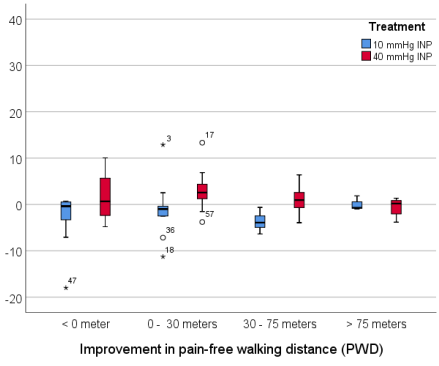


Difference between categories:

10mmHg: p = 0.911[a]

40mmHg: p = 0.288[a]

Change in myogenic function (0.007 - 0.02 Hz):

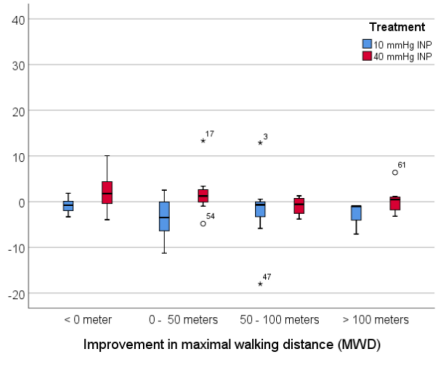


Difference between categories:

10 mmHg: p = 0.234[a]

40 mmHg: p = 0.182[a]

Change in myogenic function (0.06 - 0.2 Hz):



Difference between categories:

10 mmHg: p = 0.443[a]

40 mmHg: p = 0.551[a]

[a]Kruskal-Wallis H

Paper IV

Original research article

JVA The Journal of Vascular Access

Validation of a novel ultrasound Doppler monitoring device (earlybird) for measurements of volume flow rate in arteriovenous fistulas for hemodialysis

The Journal of Vascular Access
1–9

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Abstract

Background: Controversy exists regarding surveillance of arteriovenous fistulas for hemodialysis to increase patency. A significant reduction in volume flow rate (VFR) should lead to diagnostic evaluation and eventually intervention. Several methods are available for VFR measurements, but all of them are associated with low reproducibility. VFR trend analysis is suggested as an improved solution. It is therefore a need to find user-friendly, cost and time-effective modalities. We present a novel Doppler ultrasound device (earlybird) which could bridge this gap. It includes an easy-to-use and light-weight single element transducer.

Methods: In an experimental and clinical setting, we compared earlybird to duplex ultrasound to assess VFR. In a closed circuit of blood-mimicking fluid, 36 paired calculations of calibrated, duplex ultrasound and earlybird VFR was measured. In addition, 23 paired recordings of duplex ultrasound and earlybird VFR was measured in 16 patients with underarm arteriovenous fistulas. Pearson correlation, intraclass correlation coefficient, root-mean-square and Bland-Altman plots were analyzed.

Results: Strong correlation ($r = 0.991$, $p < 0.001$), and excellent level of agreement (ICC = 0.970 (95% CI 0.932 - 0.985), $p < 0.001$) between earlybird and the calibrated VFR was found in the experimental setup. This was confirmed in the clinical setting, with a strong correlation ($r = 0.781$, $p < 0.001$) and moderate to good level of agreement (ICC = 0.750 (95% CI 0.502–0.885), $p < 0.001$) between earlybird and duplex ultrasound VFR measured at the arteriovenous fistulas outflow veins. In the Bland-Altman plot-analysis for the experimental setup, we found smaller limits of agreement, a smaller consistent and proportional bias, as well as greater accuracy of earlybird than DUS when compared to the calibrated VFR.

Conclusion: Earlybird is a feasible tool for VFR measurements and could be a future promising device for easy assessment and surveillance of AVF for hemodialysis.

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Introduction

Controversy exists over the benefit of surveillance of vascular access.[1,2] Some guidelines recommend surveillance of arteriovenous fistulas (AVF) for hemodialysis at regular intervals to improve patency.[3–6] The European Society for Vascular Surgery (ESVS) recommend that flow measurements should be performed monthly for arteriovenous grafts and every three-month for fistulas.[3] Access flow surveillance is found beneficial for AV fistulas, but studies have not shown the same benefit for grafts.[7–9] The clinical benefit and cost effectiveness of AVF surveillance regimens are a subject of debate.[1,3,9–12] Updated guidelines from the European Renal Best Practice (ERBP) and The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) have found that the available evidence is inconclusive to make a recommendation on AVF surveillance and they do not suggest routine surveillance of grafts.[1,12]

Several volume flow rate (VFR) quantification modalities are available. Indirect VFR measurements during dialysis with dilution techniques, together with monitoring the venous pressure are routinely used.[3,4,9] Direct flow measurements with magnetic resonance imaging has the benefit of providing an anatomic overview but is less available and is regarded as costly.[9] Duplex ultrasound (DUS) is a more available tool for VFR measurement. ESVS guidelines promotes surveillance of AVF with DUS at regular intervals to reduce the risk of AVF-thrombosis.[3] Often the brachial artery is preferred for DUS VFR of the AVF,[3,13–15] but the radial artery or the AVF outflow vein could also be used. Ultrasound examinations gives at the same time a functional and anatomic view of the vessels of interest.[9]

VFR measurements are known to be sensitive to hemodynamic fluctuations in dialysis patients caused by variations in the hydration level, measurements errors and user-dependency. To cope with low reproducibility, it is proposed to frequently assess VFR and use trend analysis to select patients for further diagnostic evaluation.[1,3,12] Research in new technology to facilitate and clarify the role of monitoring and surveillance of vascular access are needed.[1,12]

Earlybird is a novel ultrasound Doppler monitoring device. A recent published study has shown that the device can detect alterations in blood flow velocities with comparable accuracy as laser Doppler flowmetry and pulsed wave Doppler.[16] Earlybird has the potential to be manufactured as a lightweight, low-cost, and easy-to-use device for blood flow monitoring. The aim of this project was to evaluate whether earlybird could be used to monitor VFR in AVF for hemodialysis and to be a potential future tool for surveillance of vascular access. The feasibility of earlybird for VFR measurements both in an experimental setup and in patients with AVF is investigated.

Material and Methods

Earlybird

Earlybird is an ultrasound Doppler monitoring device, which consists of a single element transducer (Per Kristian Bolstad, University of Southeast Norway),[17] acquisition hardware, and a user interface. The transducer was fixed in a case, that ensured a 63.3° insonation angle to the surface, Figure 1. The nominal frequency is 7.8 MHz, transmitted at a pulse repetition rate of 8 kHz. In-house software, developed in MATLAB (MathWorks® R2018a), recorded multi-gated Doppler signals for later post processing of power-Doppler M-mode and spectrogram. For VFR measurement the earlybird-probe was placed above the simulated vessel of interest or at the proximal part of the AVF outflow vein. The probe was positioned, guided by real-time Doppler spectrogram, to obtain laminar flow.

Duplex ultrasound

For DUS VFR measurements in the experimental setup we used GE Vivid E95 (General Electric (GE) Vingmed Ultrasound, Horten, Norway), equipped with 9L and 11L linear transducers, while for the clinical setup we used GE Logic S8 ultrasound system (GE Healthcare, Milwaukee, Wisconsin, USA), equipped with a ML6-15 transducer. DUS measurements were performed by an experienced ultrasound examiner. The lumen or vessel of interest was precisely visualized. Several repeated measurements were performed. Flow velocity was measured in angle corrected pulse wave Doppler-mode at an insonation angle below 60°. Sample-volume was set between 70% and 99% of the vessel lumen. Inner diameter was measured perpendicular to the vessel wall. VFR was automatically calculated by the ultrasound machine software, based on the intensity-weighted mean frequency (TAMEAN), and diameter.

Experimental setup

The experimental validation setup consisted of a peripheral flow phantom (Model 524, Peripheral Vascular Doppler flow Phantom, ATS Laboratories, Norfolk, USA),

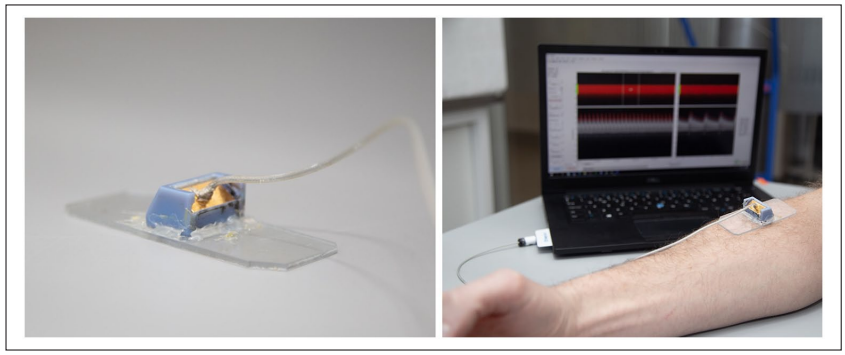

Figure 1. Prototype of earlybird probe attached to a fastening stand. In the background, interface illustrating Doppler flow velocity curves (photo: Karl Jørgen Marthinsen/NTNU).

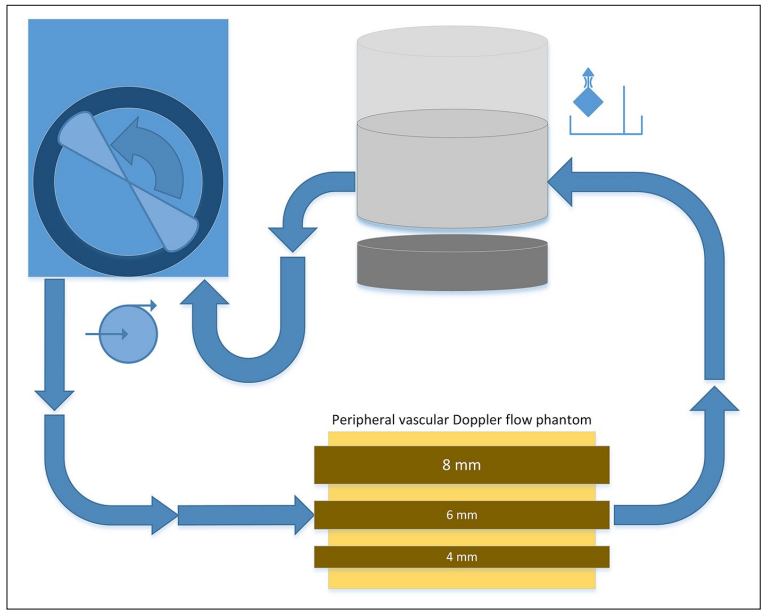

Figure 2. Experimental setup: closed loop-circuit for blood-mimicking-fluid including vented reservoir on top of a magnetic stirrer, dual-headed rotator pump and vascular phantom with three different lumen diameters of 8, 6, and 4 mm.

a reservoir and a dual head rotator pump (Multiflow Rollerpump, Stöckert Instrumente, Münich, Germany) connected in a closed circuit, Figure 2. The circuit was filled with a blood mimicking fluid, consisting of a solution of distilled water and corn starch. The peripheral flow phantom is made of a tissue-mimicking material of urethane rubber, and consists of several lumens simulating vessels, ranging from 4 to 8 mm in diameter, at 15 mm depth from the surface. The speed of sound in urethane rubber is 1450 m/s + 1.0% at 23°. We assume that the simulated vessel lies parallel to the surface and that flow is laminar and radially symmetric. By adjusting the speed of the rotator pump, different pulsatile VFR (calibrated VFR) could be moved through the phantom. The calibrated VFR was estimated by measuring the variation of the reservoir-weight using a scale and controlled for each separate measurement. We assumed the density of water to be 1 kg/dm^3. For each calibrated VFR a paired recording was made with earlybird and DUS VFR, with respectively the 9L and 11L ultrasound transducer.

Clinical setup

In total 16 patients, established with a distal radio-cephalic forearm AVF for hemodialysis access, were recruited from one dialysis unit. The median age was 66 years (range 44–87). The patients were non-fasting, and all measurements were done before cannulation of the fistula vein and connection to the hemodialysis unit.

The patients underwent a DUS-scanning of bilateral brachial arteries, the ipsilateral radial artery, and the outflow vein of the AVF. DUS VFR were calculated from all anatomic sites. The DUS VFR for the brachial artery was calculated by subtracting the contralateral against the ipsilateral achieved measurements as described in guidelines.[3] Repeated measurements were made for each vessel. At the discretion of the examiner, based on consistency of the recording, undisturbed signal acquisition and laminar flow, the recording with the most consistent measurement was chosen to be used for further analysis. We recorded flow velocities with earlybird at the AVF outflow vein as earlier described.

Earlybird volume flow rate calculation

For earlybird VFR calculations we assumed fully developed laminar parabolic flow. Calculations were made post procedural. VFR (Q) for parabolic flow, where TAV is time-averaged maximum velocity and r the radius, is given by equation (1):

$$Q = A\bar{v} = \pi r^2 \frac{TAV}{2} \quad (1)$$

Maximum velocity over the vessel cross-section was automatically calculated from the Doppler spectrogram, and corrected for differences in speed of sound between the rubber-phantom and tissue. An algorithm to automatic calculate vessel diameter (EB D) based on power Doppler M-mode data was developed, compensating for sample volume size and insonation angle. No visual confirmation of the course of the vessel are made with earlybird. We assumed that the vessel of interest lies parallel to the skin. In a clinical setting the vessel may deviate, this will lead to an imprecise estimation of diameter. Angle corrected TAV was based on an insonation angle of 63.3°. VFR expressed as equation (2).

$$Q = \pi * \left(\frac{EB\,D}{2}\right)^2 * \frac{uncorrected\,TAV}{\cos(63.3)} * \frac{1}{2} * 60\,ml/min \quad (2)$$

Statistics

Normality was assessed. For the absolute VFR-data, the relative difference between each method and the calibrated reference for experimental data, as well as the relative difference between each method and the mean of the methods for the clinical data, parametric tests were used since the relative differences were approximately normally distributed. Pearson correlation analysis are presented. Intra-rater variability and reliability analysis for agreement between methods were done using intraclass correlation coefficient (ICC) with its 95% confidence intervals, using single measurements for absolute agreement in a two-way mixed model,[18,19] and level of agreement reported according to Koo and Li.[20]

Bland-Altman plots, 95% limits of agreement, and root-mean-square (RMS) were calculated based on the relative difference of DUS and earlybird from the calibrated VFR for the experimental data and based on the relative difference from the mean of DUS and earlybird VFR at the AV outflow vein for the clinical setup.[21,22] Consistent bias expressed as the mean relative difference was assessed using One-Sample t-test. Linear regression was used to assess proportional bias. All statistical analyses were done in in SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY).

Ethics

The study was approved by the regional ethical committee (REC central Norway, 044/2017) and by the Norwegian Government of Health, June 12th, 2017. All participants provided written informed consent.

Results

Experimental setup

In an experimental setup 36 paired calculations for calibrated, earlybird and DUS VFR were recorded. Of the ultrasound examinations, 18 were taken using the 11L transducer and 18 using the 9L transducer (supplementary material; table 1).

The calibrated VFR correlated against DUS VFR ($r = 0.984$, $p < 0.001$) as well as for earlybird VFR ($r = 0.991$, $p < 0.001$), Figure 3. No relative difference between DUS and calibrated VFR was seen between the two types of transducers (1.51% (SE 8.22), $p = 0.855$). For further analysis of DUS VFR, the data of two transducers were combined. The level of agreement between methods for DUS and calibrated VFR is poor to excellent, due to a wide 95 % confident interval, ICC of 0.949 (95% CI, 0.449–0.986), $p < 0.001$. While for earlybird and calibrated VFR an excellent level of agreement was found with an ICC of 0.970 (95% CI, 0.932–0.985), $p < 0.001$.

The relative difference of the calibrated VFR to DUS and earlybird VFR were calculated and plotted in a Bland-Altman-like plot with 95% limits of agreement, Figure 4.

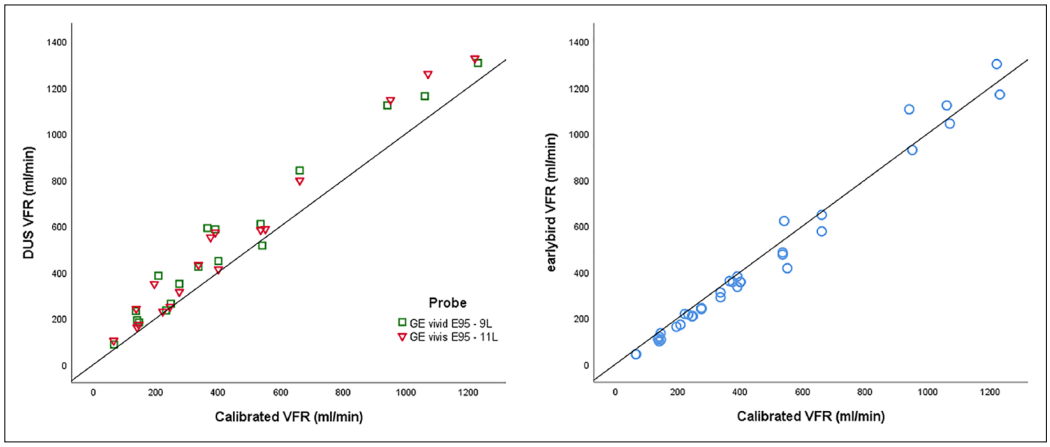

Figure 3. Scatterplot of correlation between calibrated volume flow rate (VFR) and duplex ultrasound (DUS) and earlybird VFR respectively. VFR is reported in ml/min. The 9L and 11L transducers are marked separately. Correlation analysis for DUS includes both transducers. Pearson Correlation for DUS VFR, $r = 0.984$, $p < 0.001$, and for earlybird VFR, $r = 0.991$, $p < 0.001$.

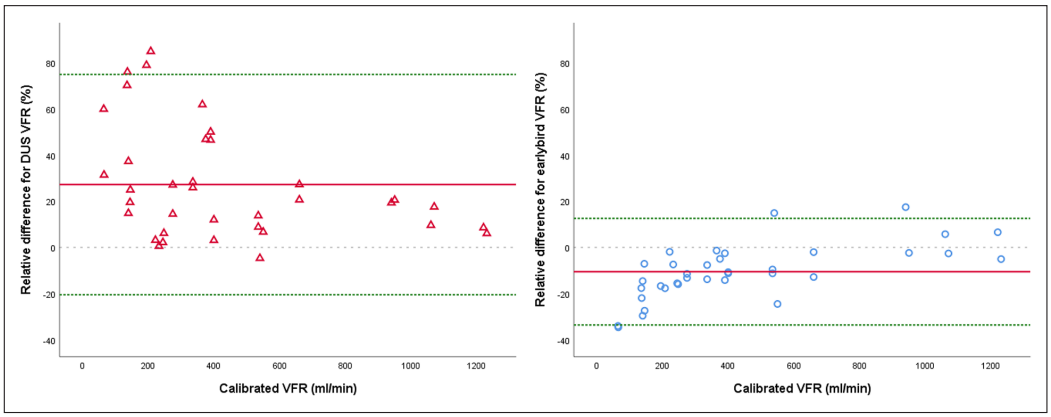

Figure 4. Bland-Altman plots of the relative difference (%) of duplex ultrasound (DUS) or earlybird, and the calibrated volume flow rate (VFR) ((VFR—calibrated VFR)/calibrated VFR). Mean relative difference (red solid line) and 95% limits of agreement (dotted green line) are given.

There is a consistent bias of the relative difference of DUS from the calibrated VFR of 27.2% (SE 4.06), $p < 0.001$, 95% limits of agreement −20.5 and 74.9%, and for earlybird VFR of −10.5% (SE 1.96), $p < 0.001$, 95% limits of agreement −33.5 and 12.6%. There is a proportional bias for both DUS VFR ($B = -0.029$, SE 0.012, $p = 0.017$) and earlybird VFR ($B = 0.023$, SE 0.005, $p < 0.001$), indicating that the relative difference changes with −0.029 and 0.023, respectively, for one unit in increase of calibrated VFR. Accuracy analysis between calibrated and DUS VFR gives an RMS-error of 36.3%, and for calibrated and earlybird VFR of 15.6%.

DUS and earlybird intra-rater reliability was analyzed for experimental data by setting the DUS measurements with the two different transducers, done on the same calibrated VFR, as two separate examinations. An excellent intra-rater reliability was found for both DUS (0.995 (0.986, 0.998), $p < 0.001$) and earlybird (0.977 (0.940, 0.991), $p < 0.001$).

Clinical experiment

In total 16 patients were recruited to undergo evaluation of VFR of their underarm AV-fistula. Of these, three patients

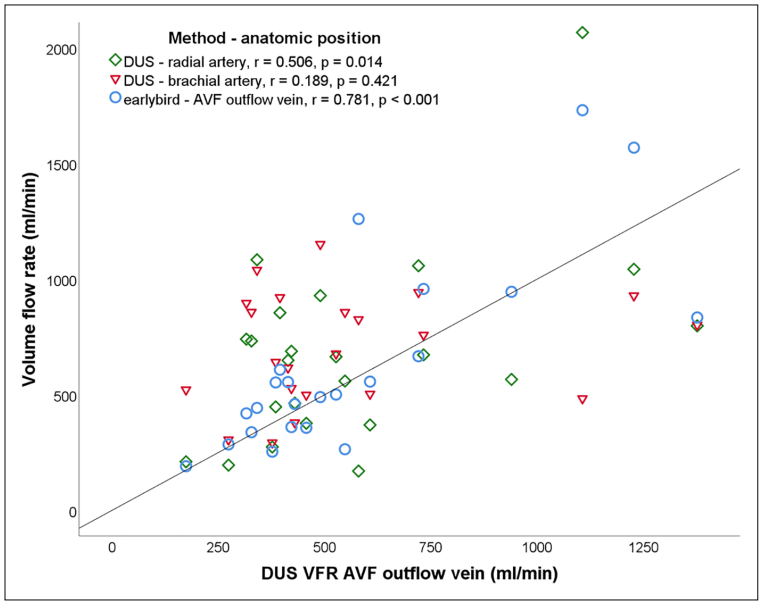

Figure 5. Scatterplot of DUS VFR of the AVF outflow vein against the DUS VFR of the radial artery (a. radialis), subtracted brachial artery (a. brachialis) and earlybird VFR positioned at the AVF outflow vein. Pearson correlation (*r*) is reported for DUS VFR of the AVF outflow vein and VFR for each method respectively. The identity line is given.

were examined twice and two patients three times at a three-month interval. Each paired measurement is regarded as one sample, making a total of 23 paired recordings of DUS and earlybird (Supplemental material; table 2). It was not possible to obtain DUS measurements of the contralateral brachial artery for one patient.

There is a strong correlation between DUS and earlybird VFR of the AVF outflow vein ($r = 0.781$, $p < 0.001$). DUS VFR of the radial artery moderately correlated with DUS and earlybird VFR for AVF outflow vein ($r = 0.506$, $p = 0.014$ and $r = 0.578$, $p = 0.004$, respectively). DUS VFR of the subtracted brachial artery did not correlate to the DUS VFR of the radial artery or the AVF outflow vein, as well as earlybird VFR for AVF outflow vein ($p = 0.101$, $p = 0.400$, $p = 0.421$), Figure 5. The level of agreement for DUS and earlybird VFR of the AVF outflow vein is moderate to good, with an ICC of 0.750 (95% CI 0.502, 0.885), $p < 0.001$.

In the clinical setup, we compared two methods of estimating VFR based on the Doppler-technique, both methods are suspected to divert from the "true" VFR. Therefore, mean VFR of the two methods were calculated and used for further analysis, presented in a Bland-Altman plot in Figure 6. There is no significant relative difference from the mean VFR of the two methods, 2.9% (SE 3.39), $p = 0.399$, indicating no consistent bias. No proportional bias was found ($B = 0.012$ (SE = 0.010), $p = 0.245$). The 95% limits of agreement are −29.0 and 34.8%. RMS-deviation for the relative difference from the mean of DUS and earlybird VFR is 16.2%.

Discussion

We tested a novel ultrasound Doppler device (earlybird) against ultrasound to determine VFR. In an experimental setting, both earlybird and DUS estimates the calibrated VFR well. We found greater accuracy and less bias for VFR estimated with earlybird than for DUS. Repeated measurements with earlybird were consistent. The findings were confirmed in a clinical setting, where we compared earlybird and DUS VFR at the AVF outflow vein.

Both in the experimental and clinical setup, DUS and earlybird VFR deviates from the calibrated and mean VFR. The "true" VFR in a clinical setting is not known. In a simulator-model analyzing accuracy of DUS VFR measurements of AVF outflow vein, the measured VFR deviated 35 ± 36%.[23] An error of DUS VFR measurements of approximate 30% are reported in clinical settings.[3,24] In AVF surveillance programs, a reduction in VFR less than 33% should not be regarded as significant.[3] Trend analysis has been proposed to overcome these well-known obstacles.[3]

Indirect VFR measurements by dilution techniques or DUS have been the method-of-choice to evaluate dialysis

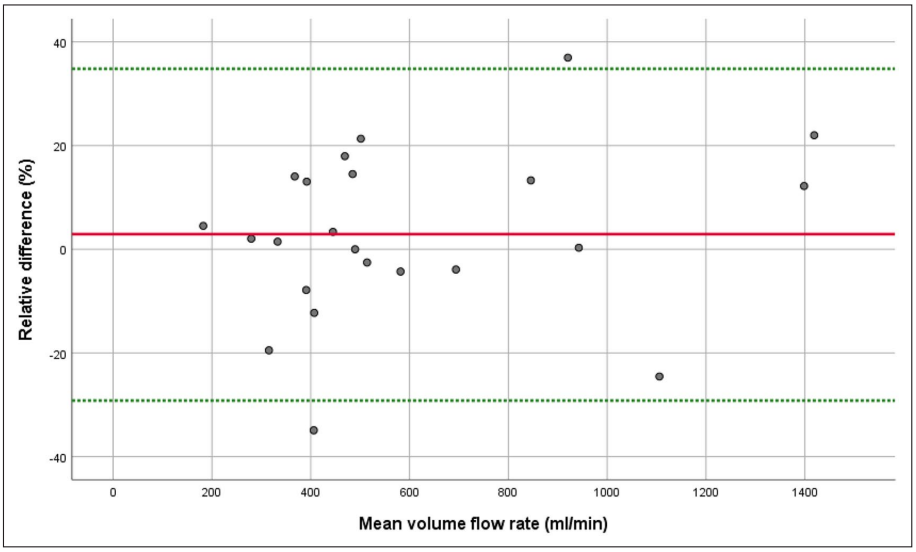

Figure 6. Bland-Altman plot of the relative difference (%) of DUS and earlybird VFR from the mean VFR ((earlybird VFR—mean VFR)/mean VFR), measured at AVF outflow vein. Mean relative difference (red solid line) and 95% limits of agreement (dotted green line) are given.

access functioning. There is good agreement between the methods.[25–27] These methods need specialized trained personnel, often require flow reversal during dialysis and can be time consuming.[9] Because of high technical failure-rate and high-cost of dilution techniques, DUS is the recommended method.[25] In the early development of ultrasound, noninvasive blood flow measurements of AVF and grafts for hemodialysis were explored. Rittgers et al.[28] used a 5 MHz flat-head probe with a fixed angle of 60° to the surface to evaluate access grafts with a known diameter. The method was found to be useful, safe and repeatable. Bouthier et al.[29] described a series of 32 patients where AV-fistula blood flow was non-invasive evaluated using a double transducer probe, which made it possible to correct for angle and calculate diameter. Easy-to-use and low-cost devices could allow patients and non-specialized healthcare workers to perform VFR trend surveillance of AV-fistulas daily or at every dialysis visit. This would allow for frequent VFR-data collection, and could be combined with that is, weight and blood pressure, further research is needed to evaluate whether this could change the surveillance practice.

Measurements of flow velocities by the Doppler method is user dependent, needs accurate angle correction and is highly dependent of the insonation angle.[30–32] The error of the selected angle should be less than two degrees to achieve a measurement error of 10%. For calculation of earlybird VFR we have assumed that the vessel lies parallel to the surface. In the clinical setup it is not given that the AVF outflow vein is accurately parallel, especially when the vessels enter the antecubital fossa where it lies in more subcutaneous fat and deflects downwards. We have not corrected for this, and this can result in inaccurate earlybird VFR, due to imprecise calculation of both Doppler velocity and diameter. The AVF outflow vein may have considerably tortuosity, diameter alterations and be close to the anastomotic area, which all contribute to less developed parabolic flow.[33] This lowers the accuracy of VFR calculations. These concerns are great limitations of earlybird.

Different anatomic sites for DUS examination of AVF-functioning are described. DUS measurements of VFR at the brachial and radial artery, as well as AVF outflow vein are commonly used.[8,13,34,35] Existing guidelines have not described the optimal anatomic site,[3,4,36] although the brachial artery is often preferred.[14,15,35] Its anatomic position and easy access facilitate an insonation angle $< 60°$, as well as it more often presents with laminar and radial symmetric flow. Other studies have found a strong correlation between VFR at the AVF outflow vein and the brachial artery.[35] In our small clinical sample, we did not find the same correlation. This may be due to anatomic variations, that is, side-branches to the fistula which divert blood flow away from the vessel used to measure VFR, and difference in flow through the ulnar artery and palmar-arcade. Of the included paired clinical measurements, one sample deviates from the expected values with a relative low VFR (484 ml/min) in the brachial artery and a fourfold VFR

(2067 ml/min) of the radial artery (No. 21; Supplemental material). This may be due to erroneous measurements or under- or over-estimating VFR in the brachial or radial artery, respectively. We chose not to exclude this sample, since this may represent real-life challenges in VFR estimation. In our study, AVF outflow vein was chosen for earlybird VFR measurements because of its easy access and visibility.

The Bland-Altman plots reveal an increasing relative difference in the DUS VFR-measurements for low calibrated VFR in an experimental setting. This may be due to the wall-filter which remove low-velocity signals and increase the calculated DUS VFR, especially at low velocities. Spectral broadening due to transit time, can cause overestimated velocity. The effect will be less in a unfocused single element probe than in a linear probe, mainly due to wider beamwidth.[37] For earlybird the overestimation is less than 5%. To minimize user-dependent error of earlybird measurements, an automatic algorithm-based calculation of diameter and velocity were performed. The algorithm is based on known instrument settings and transducer geometry and is not built on or adapted by the results of this study. Errors in diameter assumptions will greatly influence VFR-calculations. The experimental VFR-measurements confirms an adequate algorithm for calculation of diameter and velocity.

Intra or inter-observatory changes are well-known sources for errors in the DUS measurement[31] and was already described when Gill[38] for the first time described VFR as a possible technique for blood flow evaluation. In the experimental part of this study, we found an excellent intra-observer reliability. We did not perform an inter-rater analysis. Exploration of these sources of error are outside the scope of this proof-of-concept study, but to further assess intra- and inter-observatory reliability would be interesting to validate earlybird as a device for AVF surveillance.

Conclusion

Our study indicates that earlybird is a feasible tool for VFR measurements. Technical development may increase user-friendliness and its clinical applicability and earlybird may be a future promising device for easy assessment and surveillance of hemodialytic vascular access.

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Author contributions

Conception and design: EMP, SF, HT, AS. Data collection: EMP. Analysis and interpretation: EMP, JA, ØS, HT, AS. Statistical analysis: EMP, JA, ØS. Writing the article: EMP. Final approval and critical revision of the article: EMP, JA, SF, ØS, JH, HT, AS.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article, except Hans Torp who is the inventor of earlybird and shareholder of CIMON Medical AS. CIMON Medical AS was not involved in the study design; collection, analysis, or interpretation of data; manuscript writing; or the decision to submit the manuscript for publication.

Ethical approval

The study was approved by the regional ethical committee (REC central Norway, 044/2017) and by the Norwegian Government of Health, June 12th, 2017. All participants provided written informed consent.

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Supplemental material

Supplemental material for this article is available online.

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Supplementary material: Pettersen, EM et al, Validation of a novel ultrasound Doppler monitoring device (earlybird) for measurements of volume flow rate in arteriovenous fistulas for hemodialysis.

TABLES

Table 1: Volume flow rate (VFR) experimental setup

	Calibrated		**Duplex ultrasound**			**earlybird**	
	Diameter (mm)	**VFR (ml/min)**	Probe	Diameter (mm)	**VFR (ml/min)**	Diameter (mm)	**VFR (ml/min)**
1	4.0	**65**	11L	3.9	**104**	3.6	**52**
2	4.0	**66**	9L	3.7	**87**	3.4	**46**
3	6.0	**136**	9L	5.8	**231**	5.4	**116**
4	6.0	**137**	11L	5.9	**241**	5.4	**113**
5	4.0	**140**	11L	3.7	**161**	4.2	**145**
6	4.0	**140**	9L	4.0	**192**	4.1	**133**
7	4.0	**145**	11L	3.8	**173**	4.1	**147**
8	4.0	**146**	9L	3.8	**182**	4.0	**144**
9	8.0	**195**	11L	7.9	**349**	6.7	**152**
10	8.0	**208**	9L	7.8	**385**	7.0	**174**
11	4.0	**222**	11L	3.8	**229**	4.3	**249**
12	4.0	**233**	9L	3.8	**234**	4.2	**234**
13	4.0	**245**	11L	3.9	**250**	4.4	**275**
14	4.0	**248**	9L	3.9	**263**	4.4	**275**
15	6.0	**275**	11L	5.8	**315**	6.1	**274**
16	6.0	**275**	9L	5.8	**350**	6.2	**279**
17	6.0	**336**	11L	5.9	**431**	6.1	**325**
18	6.0	**336**	9L	5.9	**423**	6.2	**331**
19	8.0	**365**	9L	7.9	**591**	8.0	**403**
20	8.0	**375**	11L	7.9	**550**	7.9	**401**
21	8.0	**390**	11L	7.9	**571**	7.9	**385**
22	8.0	**390**	9L	7.9	**585**	8.2	**417**
23	6.0	**400**	11L	5.8	**412**	6.2	**402**
24	6.0	**400**	9L	5.8	**448**	6.3	**406**
25	6.0	**535**	11L	6.0	**582**	6.4	**538**
26	6.0	**535**	9L	5.9	**609**	6.4	**538**
27	6.0	**540**	9L	5.9	**515**	6.9	**646**
28	6.0	**550**	11L	5.9	**587**	6.0	**474**
29	8.0	**660**	11L	7.9	**797**	8.7	**687**
30	8.0	**660**	9L	7.9	**840**	8.7	**708**
31	8.0	**940**	9L	8.0	**1122**	9.5	**1223**
32	8.0	**950**	11L	8.0	**1145**	9.3	**1152**
33	8.0	**1060**	9L	7.9	**1162**	9.3	**1212**
34	8.0	**1070**	11L	7.9	**1258**	9.4	**1236**
35	8.0	**1220**	11L	8.0	**1325**	9.5	**1412**
36	8.0	**1230**	9L	8.0	**1305**	9.5	**1483**

Table 2: Volume flow rate (VFR) clinical setup

Anatomic position	Duplex ultrasound						earlybird	
	Ipsi Brachial artery	Contra Brachial artery	SUM Brachial artery	Radial artery		**AVF outflow vein**		**AVF outflow vein**
No	VFR (ml/min)	VFR (ml/min)	VFR (ml/min)	VFR (ml/min)	Diameter (mm)	**VFR (ml/min)**	Diameter (mm)	**VFR (ml/min)**
1	569	47	522	210	4.5	**174**	4.2	**191**
2	400	95	305	196	4.7	**274**	4.6	**285**
3	951	54	896	741	4.9	**316**	5.4	**419**
4	913	55	858	732	3.9	**328**	4.0	**338**
5	1251	212	1039	1084	5.1	**341**	5.7	**443**
6	342	50	293	274	6.1	**377**	4.8	**254**
7	682	40	641	448	6.2	**385**	6.2	**553**
8	1126	205	921	855	5.7	**395**	6.4	**608**
9	701	84	616	649	6.4	**414**	6.3	**555**
10	561	33	528	688	7.0	**422**	6.4	**360**
11	479	100	379	464	5.2	**430**	6.6	**460**
12	617	118	499	377	6.0	**457**	5.4	**357**
13	1322	171	1151	929	5.4	**490**	5.0	**490**
14	766	89	677	664	6.6	**527**	6.6	**501**
15	945	88	857	559	5.8	**548**	4.6	**264**
16	1041	216	825	170	8.8	**580**	11.1	**1261**
17	517	14	503	369	5.3	**607**	5.7	**557**
18	1066	123	943	1058	6.7	**721**	6.0	**667**
19	894	136	758	673	5.7	**733**	7.2	**958**
20	750	-	-	566	6.5	**940**	8.3	**945**
21	484	1	484	2067	6.5	**1107**	9.2	**1731**
22	1123	194	929	1043	5.5	**1228**	8.6	**1569**
23	889	88	802	798	8.0	**1377**	6.2	**834**

SUM Brachial artery VFR is calculated from subtracting the contralateral brachial artery from the ipsilateral brachial artery. Table is sorted after AVF outflow vein.

Congress abstract I

Earlybird - A Novel Ultrasound Doppler Monitoring Device - Potential Future Application In Per-Operative Monitoring

Miscellaneous

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Introduction: Peripheral arterial disease (PAD) is estimated to affect 200 million people worldwide [1]. Critical limb threatening ischaemia (CLTI) is the end stage of PAD and requires prompt and adequate revascularisation to prevent limb loss [2]. No objective method to evaluate whether a limb is adequately revascularised is widely adopted in a clinical setting [3]. Earlybird, a novel ultrasound Doppler monitoring device [4, 5], may bridge this gap.

Establishment of in line flow to the foot and relevant angiosome, appearance of "wound blush", flow characteristics and improvement of arterial calibres are used to evaluate peri-procedural success. Per-operative monitoring with transcutaneous oxygen tension ($TcPO^2$), skin perfusion pressure (SPP), perfusion or fluorescence angiography, tissue oxygen saturation mapping and duplex ultrasound flow measurements, are of potential benefit [3].

By adapting basic ultrasound technology, one of the authors (HT) has developed a novel ultrasound Doppler monitoring device. Earlybird is a small, lightweight ultrasound probe, which can be gently fixed to the patient's skin. It is unique because of its potential to simultaneously and continuously measure peripheral blood flow velocity from 2mm to 40 mm depth, a range involving the epidermis, dermis, hypodermis as well as musculature. In a recent published study, we found that Earlybird correlates well with laser Doppler flowmetry (LDF) and pulsed Doppler to assess microcirculatory function in healthy subjects [4]

A future potential of Earlybird could be per-operative monitoring to assess adequate limb revascularisation. To determine whether Earlybird could detect change in blood velocities during an endovascular procedure a pilot study was performed.

Methods: An Earlybird probe was attached over the posterior tibial artery at the medial malleolus of the treated limb. Several successive intermittent recordings were done of different duration, ranging from 7 seconds to 15 minutes. Per-operative flow velocities were continuously recorded. Real time processing and post processing were performed by in house software, developed in MATLAB (MathWorks ® R2018a). The study was approved by the regional ethical committee.

Results: An 86 year old female presented with rest pain (Fontaine III, Rutherford 4, WIfI 2) in her right leg over the last month. CT angiography revealed an occlusion of her right external iliac artery. A digital subtraction angiogram confirmed the findings. The lesion was passed by cross over with a catheter and predilated. A stent (S.M.A.R.T Control, Cordis) was placed, followed by balloon dilation.

Mean velocity (v_{mean}) before the intervention was found to be 4.58 cm/s. After revascularisation measurements showed an increase to $v_{mean} = 7.81$ cm/s, which is an increase of v_{mean} of 3.23 cm/s (171 % of baseline).

Conclusion: Earlybird detects changes in flow velocities during an endovascular revascularisation procedure. Further investigations are needed to assess threshold values and evaluate Earlybird's monitoring capabilities in predicting patient outcome. Earlybird could be a future tool for clinical decision making during endovascular treatment. **Disclosure:** Nothing to disclose

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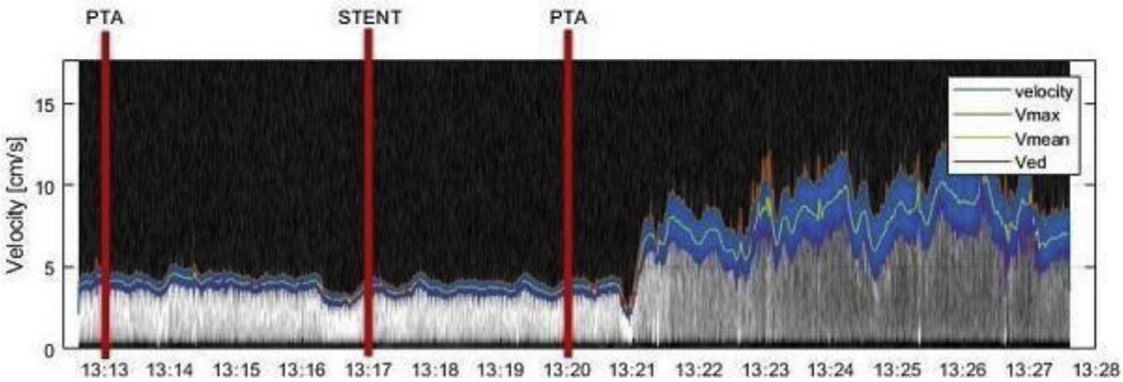

Figure: Velocities during endovascular treatment]

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