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Complete List of Authors:	Simons, Michelle; University Medical Centre Utrecht Department of Vascular Medicine, Vascular Medicine Groen, Marijn; University Medical Centre Utrecht Department of Cardiology, Cardiology de Borst, Gert-Jan; University Medical Centre Utrecht Speciality Surgery, Vascular Surgery Leiner, Tim; University Medical Center Utrecht Imaging Division, Radiology Doevendans, Pieter; University Medical Centre Utrecht Department of Cardiology, Cardiology; Netherlands Heart Institue Ebbini, Emad; University of Minnesota College of Science and Engineering, Electrical and computer Engineering van Es, René; University Medical Centre Utrecht Department of Cardiology Hazenberg, Constantijn; University Medical Centre Utrecht Speciality Surgery, Vascular Surgery
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Robot-assisted high intensity focused ultrasound therapy for the treatment of atherosclerotic plaques in the femoral artery: protocol for a pilot study

M.V. Simons[1], M.V.Simons-4@umcutrecht.nl, M.H.A. Groen[2], M.H.A.Groen-4@umcutrecht.nl, G.J. de Borst[1], G.J.deBorst-2@umcutrecht.nl, T. Leiner[5], T.Leiner@umcutrecht.nl, P.A.F. Doevendans[2, 3, 4], P.Doevendans@umcutrecht.nl, E.S. Ebbini[6], ebbin001@umn.edu, R. van Es[2], R.vanEs-2@umcutrecht.nl, C.E.V.B. Hazenberg[1], C.E.V.B.Hazenberg@umcutrecht.nl

[1] Department of Vascular Surgery, University Medical Center Utrecht, Utrecht, the Netherlands.

[2] Department of Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands.

[3] Central Military Hospital, Utrecht, The Netherlands.

[4] Netherlands Heart Institute, Utrecht, The Netherlands.

[5] Department of Radiology, University Medical Center Utrecht, Utrecht, The Netherlands.

[6] Department of Electrical and Computer Engineering, University of Minnesota, Minneapolis, MN, USA

Corresponding author: M.V. Simons, M.V.Simons-4@umcutrecht.nl, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, telephone: +31 6 39034914

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ABSTRACT

Introduction: Peripheral arterial disease (PAD) is an atherosclerotic disease leading to stenosis and/or occlusion of the arterial circulation of the lower extremities. The currently available revascularization methods have an acceptable initial success rate, but the long-term patency is limited, while surgical revascularization is associated with a relatively high perioperative risk. This urges the need for development of less invasive and more effective treatment modalities. This protocol article describes a study investigating a new non-invasive technique that uses robot assisted high intensity focused ultrasound (HIFU) to treat atherosclerosis in the femoral artery.

Methods and analysis: A pilot study is currently performed in 15 symptomatic PAD patients with a significant stenosis in the common femoral (CFA) and/or proximal superficial femoral artery (SFA). All patients will be treated with the dual-mode ultrasound array (DMUA) system to deliver imaging-guided high-intensity focused ultrasound (HIFU) to the atherosclerotic plaque. Safety and feasibility are the primary objectives assessed by the technical feasibility of this therapy and the 30-day major complication rate as primary endpoints. Secondary endpoints are angiographic and clinical success and quality of life.

Ethics and dissemination: Ethical approval for this study was obtained in 2019 from the Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands. Data will be presented at national and international conferences and published in a peer-reviewed journal.

Trial registration numbers: Netherlands Trial Register, NL7564.

Keywords: Atherosclerosis, focused ultrasound, peripheral arterial disease, plaque debulking, thermal therapy

ARTICLE SUMMARY

This article presents the working protocol of high-intensity focused ultrasound therapy using a dual-mode ultrasound array, for transcutaneous treatment of atherosclerosis in the femoral arteries.

Strengths and limitations of this study

- Robot assisted high intensity focused ultrasound therapy is a novel concept for non-invasive treatment of atherosclerotic lesions.
- This paper describes the protocol of a first-in-human study investigating the safety and feasibility of targeting atherosclerotic plaque with high intensity focused ultrasound.
- This is a non-randomized single center pilot study. The protocol and data will be used to set up a future multicenter efficacy trial comparing conventional interventional treatment with HIFU.
- Effect of HIFU on calcification is unknown, so for this reason highly calcified lesions are not targeted in the currently running clinical trial. Consequently, the data collected in this study cannot be extrapolated to the full spectrum of plaque morphology.

INTRODUCTION

Peripheral arterial disease (PAD) is an atherosclerotic disease leading to stenosis and/or occlusion of the arterial circulation of the lower extremities. The severity of PAD varies from asymptomatic stenosis to limb-threatening ischemia.[1–3] In case of severe symptoms, revascularization is indicated. Three main options are generally available for revascularization, namely, open, endovascular or a hybrid intervention. The open option is the surgical removal of the atherosclerotic plaque (endarterectomy), or bypass surgery, where a new conduit of blood flow is created to overcome the stenosis or occlusion. The endovascular option is a minimally invasive manner of increasing the lumen diameter by inflating a balloon (angioplasty) combined with expanding a metal scaffold (stent), if indicated. In a hybrid case, open surgery and endovascular surgery are combined. The predicted benefit for patients should outweigh the potential risks and durability of the intervention.[4–6] Most peripheral arterial interventions have an acceptable initial technical and clinical success rate, but the long-term patency is limited.[5] Furthermore, fluoroscopy exposure during these procedures also possesses potential health risks, such as DNA damage and long-term health effects, to both the patient and the treatment team.[7–9]

Guidelines including The Trans-Atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC) generally recommend for revascularization options (open, endovascular or hybrid procedures) based on the anatomical location and extent of the arterial lesions. [4,5,10] Endovascular revascularization is generally preferred over open procedures due to the reduced perioperative morbidity and shorter hospital stays.[1,11] However, endovascular strategies for stenotic lesions located in anatomically ‘‘hostile’’ arterial segments or ‘‘no stent areas,’’ such as arterial flexion points (surrounding joints), remain a subject for debate. For example, the endovascular approach has not been adopted widely in the common femoral artery (groin). Despite the reduction of wound related complications and shorter hospital stay, endovascular revascularization in the common femoral artery is associated with a lower patency and increased rates of subsequent revascularization procedures. Another concern is that stent placement in this region may limit access options for future procedures, and the bending forces may cause stent fracture and subsequent arterial occlusion.[12] For this reason, despite the risk of infection and septic bleeding, open endarterectomy of the common femoral artery is still considered the gold standard therapy.[13,14]

An alternative to the increased complication risk of the open surgical approach is endovascular plaque debulking. With plaque debulking, the plaque is removed and reduced rather than cracked and pressed against the arterial wall, which might lessen vessel barotrauma and reduce the risk of plaque recoil. Directional, rotational, orbital, and laser atherectomy are current examples of endovascular plaque debulking with good technical success rates.[15] Nevertheless, the reported restenosis and subsequent reinterventions rates are comparable to regular endovascular revascularization.[15]

Noninvasive plaque debulking techniques may overcome the risk of perioperative complications associated with surgery altogether. High-intensity focused ultrasound (HIFU) enables noninvasive tissue ablation.[16–18] Dual-mode ultrasound arrays (DMUA) offers the possibility of simultaneous HIFU targeting and ultrasound (US) imaging, thereby allowing inherent registration between imaging and treatment location.[19–21] HIFU/DMUA therapy can be used for transcutaneous noninvasive ablation of targets with submillimeter precision and accuracy.[17,19–24] The pathophysiology of atherosclerosis suggests that atherosclerotic plaques may respond to HIFU/DMUA therapy.[21] It is hypothesized that the local thermal effect of HIFU causes coagulation necrosis affecting the plaque and inducing a new immune response with a complex cascade of cellular reactions, eventually leading to a decrease in plaque volume and an increased lumen diameter.[25,26]

A clinical trial currently being conducted at the University Medical Center Utrecht (UMCU) is investigating the safety and feasibility of this technique (Netherlands Trial Register trial number NL7564). This article describes the working protocol for the treatment of femoral arterial disease with this specific intervention.

Technical background: HIFU/DMUA synthesizer

The DMUA transducer used in this study is specifically developed for arterial plaque debulking purposes.[20] The 3.5-MHz 64-element concave shaped dual mode array transducer (Imasonic, Voray sur l'Ognon, France) uses the spherical shape for mechanical steering of the US beams (Table 1). The transducer is attached to a six degrees-of-freedom robotic arm (UR3, Universal Robots, Odense, Denmark) to allow precise positioning that enables accurate imaging and treatment on a sub-millimeter scale.

The HIFU/DMUA synthesizer is an integrated medical device that delivers US-guided HIFU therapy (figure 1). The DMUA allows real-time monitoring of lesion formation and can therefore be used as a closed-loop feedback to control energy delivery to optimize treatment. HIFU therapy is based on the fact that US beams are partially converted into thermal energy when travelling through tissue. At low intensities, as with diagnostic US, this phenomenon does not interfere with normal physiology. However, when emitted at high intensities, US beams can produce a significant rise in temperature, leading to coagulation necrosis. With HIFU therapy, the high-intensity US beams are focused on diseased tissue, thereby producing localized submillimeter thermal lesions at the targeted tissue leading to coagulation necrosis. For safety measures, the HIFU/DMUA synthesizer is equipped with a closed-loop prescriptive image-guided control (CLC) algorithm. The energy delivery is monitored along the acoustic path of the beam to avoid affecting non-targeted tissues and to modulate the amplitude of the HIFU pulse based on image feedback. This feedback consists of single transmit focus (STF) imaging data that are gathered before and after every 10 milliseconds during the HIFU bursts.[20] STF imaging is a method of high-speed imaging enabling monitoring of the changes at the treatment site.[20,27]

Table 1 DMUA technical specifications

Type	Linear array transducer
Number of channels	2 rows of 32 elements
Mechanical focusing	Spherical
Radius of curvature	50 mm ±2 mm
Inter-element spacing	0.2 mm
Height of the elements	34 mm divided into two rows (equal height)
Frequency	3.5 MHz

Figure 1 HIFU/DMUA set-up as used during a clinical procedure

METHODS AND ANALYSIS

Study objectives

To investigate the safety and feasibility of HIFU/DMUA therapy for the treatment of symptomatic patients with atherosclerotic plaques in the femoral artery.

Study design

The study is designed as a first-in-human pilot study that is currently being conducted by the University Medical Center Utrecht with a cohort of 15 patients diagnosed with PAD. All patients are asked to provide written informed consent. After informed consent is signed, patients will undergo a thorough screening to assess eligibility for inclusion.

Primary study parameters

The main endpoints are the safety and feasibility of this experimental treatment. Safety is determined by the 30-day major complication rate. This is a composite safety endpoint, based on previously validated safety endpoints for symptomatic peripheral arterial disease.[28–30] It consists of the 30-day major adverse event rate, which is defined as any complication that requires endovascular revascularization, open revascularization, or amputation in the target limb. Secondly, it consists of the 30-day mortality rate. Feasibility is defined as being able to accomplish the HIFU/DMUA procedure to target the atherosclerotic plaque in the CFA and/or SFA.

Secondary study parameters

Secondary endpoints are defined as technical, vascular imaging, clinical, and quality of life parameters. Technical success is defined as successful visualization of the target lesion and successful delivery of HIFU therapy to the intended target lesions. During the procedure, procedural data of the device, the number, intensity, and duration of all HIFU shots delivered will be recorded and analyzed. The intensity of the shots is depending on the HIFU amplitude and tissue attenuation and absorption but is generally around 6.25 kW/cm^2.[21] After the HIFU procedure the number of shots and the efficacy of the shots will be determined. The efficacy will be analyzed based on the echogenicity change during the shot. Shots reaching threshold will be considered as effective HIFU shots.

Follow up by vascular imaging is performed with both MRI and duplex ultrasound (DUS) to visualize stenosis characteristics over time. With MRI changes in lesion length (mm), stenosis severity (%), total plaque volume (mm^3), total vascular wall volume (mm^3), and atherosclerotic (peri-) plaque characteristics like presence of edema, calcification, intraplaque hemorrhage and presence of a lipid rich necrotic core can be analyzed. DUS imaging will be used to assess the hemodynamic changes by measuring the peak systolic velocity ratio (PSV ratio) at the target lesion. Furthermore, plaque (surface) characteristics will be described and special attention will be given to assess the occurrence of arteriovenous fistula.

Clinical endpoints are assessed on functional exercise and the ankle-brachial index (ABI) measurements. Hemodynamic success is defined as a ≥ 10% improvement in the ABI at 30 days post procedure compared to baseline. Functional success is defined as a ≥ 10% improvement on exercise testing. An improvement of symptoms is also taken into account.

Patients and eligibility criteria

The present safety and feasibility study is a pilot being conducted by the University Medical Center Utrecht, the Netherlands. To determine whether HIFU treatment is safe in this study, the safety outcome of HIFU-treatment has to be non-inferior to the safety outcome of the common femoral endarterectomy (CFE). The complication rate of a CFE varies between 6 – 26 %[13], with the point of gravity around 8% (1 in 12.5 patients). Therefore, a sample size of 15 patients is chosen for this safety trial for the investigational therapy. The eligibility criteria are represented in table 2. Written informed consent is obtained during the baseline visit after a reflection period of at least 7 days after the first study information visit.

Table 2 Eligibility criteria

Inclusion criteria	**Exclusion criteria**
Patient < 85 years	Patient is diagnosed with early onset PAD
Patient is diagnosed with symptomatic PAD (ABI <0.90)	Contraindication for MRI or gadolinium contrast agent

Primary non-stented target plaque with focal stenosis in CFA/proximal SFA	Recent cardiovascular event or major surgery (<6 months ago)
Presence of lower extremity CTA imaging to measure: - Grade of stenosis 50-90% - Plaque length ≤40mm - Grade of calcification ≤50% - Distance dorsal vessel wall to the skin ≤35 mm	Contra-indication for antiplatelet therapy
Target vessel visible with DMUA/HIFU synthesizer imaging	

Data handling

Participant UMCU healthcare data, data from medical interviews, quality of life questionnaires and data from various medical tests and measurements are filed as source data in the electronic patient records. All study-related results from medical interviews, tests and measurements are collected in an electronic Case Report Form (eCRF) with audit trial functionality. Internal auditing is performed and monitoring is performed by the Julius Clinical Research (Zeist, The Netherlands). Research data is stored anonymized and personal data is stored separately. A separate file is created to identify the patient via their unique study number. Research data will be archived for 15 years after the study has ended. To be able to reproduce the study findings, and to help future users understand and reuse data, all changes made to the raw data will be documented in data queries generated in the eCRF.

Study procedures

Screening procedure

The vascular surgeon identifies potential eligible study participants. If the patient consents and meets eligibility criteria as mentioned in table 2 the HIFU specific plaque characteristics (arterial depth, grade of calcification and stenosis severity) will be evaluated in collaboration with a designated expert radiologist. Based on lower extremity computed tomography angiography (CTA) and DUS the

calcification grade of maximal 50% in the culprit lesion and arterial depth of maximal 35 mm will be measured (table 2).

Baseline visit

During the baseline visit, patients are asked to sign the informed consent form. After this a clinical interview, physical examination, DUS and magnetic resonance imaging, exercise test and a HIFU/DMUA visibility test is performed. The results of the baseline visit determine if a patient is definitively eligible for study participation.

Intervention

The patient is lying in supine position under sedation during the HIFU procedure. DUS is used initially to visualize and mark the target area on the skin before the HIFU procedure. Anatomical landmarks, such as the CFA/SFA bifurcation, proximal and distal end of the plaque, and calcifications, are marked on the skin as additional guidance for the treatment team during imaging with the DMUA transducer. After this the DMUA transducer is placed in the groin region from where the CFA/SFA plaque can be followed from proximal to distal and eventually targeted. Compared to conventional DUS, image resolution of the DMUA transducer is inferior (axial resolution is ~2.6 mm and lateral resolution is ~1.2 mm whilst conventional DUS can reach submillimeter resolution depending imaging settings)[27,31]. For this reason, additional DUS is required in this phase to ensure correct targeting.

The transducer is attached to a six-degrees-of-freedom robotic arm to ensure precise positioning and accurate imaging of the target area. The transducer is placed in the groin, perpendicular to the CFA to create a transversal plane through the artery. When the CFA is visualized, the robot arm is used to move the transducer along the vessel pathway to visualize the CFA bifurcation and proximal part of the SFA. During this visualization step, synthetic aperture (SA) imaging[27] is used for real-time visualization of the full trajectory. When the full trajectory is visualized and the target planes are determined, the HIFU therapy can start. Per transversal plane (figure 2) multiple shots will be delivered in the stenosis area with an inter-target distance of 1 mm. When a full plane is targeted, the robot moves the transducer 1mm to repeat the process of targeting in the next transversal plane (figure 2). This process is repeated for 10 planes and after this the transducer is moved up to visually inspect the skin region for eventual

thermal damage. When there is no sign of thermal damage the transducer can be moved back to the position and the therapy will be continued.

Figure 2 Panel A: Schematic overview of target planes in a vessel. Panel B: Screenshot of the DMUA/HIFU image of the vessel with target area marked by the red dot. The green box represents the skin interface and the yellow box represents the region of interest (ROI). Panel C: schematic overview of the screenshot shown in panel B.

When the stenosis is treated in full length, the vascular surgeon checks for acute vascular patency with DUS. The skin is again inspected for thermal damage and the lower extremity is assessed for any signs of distal thromboembolism.

Follow-up

The overall duration of the follow-up for study participants is three months at specific moments in time. Full follow-up scheme with associated tests and measurements is visible in table 3. During the follow-up patients are assessed on change in symptoms and eventual changes in the stenosis severity based on different imaging modalities (DUS, MRA). Hemodynamic changes are assessed based on the ABI and exercise test.

	Baseline	**Procedure day**	**+1 day**	**+7 days**	**+14 days**	**+21 days**	**+30 days**	**+90 days**
Standardized Medical interview	+	+	+	+	+	+	+	+
Physical examination	+	+	+	+	+	-	+	+
Ankle brachial index	+	+	+	+	+	-	+	+
MRA	+	-	+		-	-	+	-
DUS	+	+	+	+	+	-	+	+
Exercise test	+	-	-	-	-	-	+	-
Quality of life questionnaire	+	-	+	-	+	-	+	+

Table 3 Follow-up scheme for study participants. The + sign indicates the test is performed during the visit.

Adverse events

In case of an adverse event (AE) the research team will convene and discuss this within a week, to evaluate whether the AE was procedure-related and to evaluate the safety of the HIFU-treatment. Two independent experts will periodically (every 5 patients) review all AEs. In case of a serious adverse

event (SAE), the independent experts is informed <7 days. In case of life-threatening adverse events or death, the independent experts is informed <3 days.

SAE's are reported through the web portal '*ToetsingOnline*' to the accredited Medical Ethics Committee that approved the protocol and to the *'Inspectie gezondheidszorg en jeugd (IGJ)*', within 15 days after first knowledge of the SAE. SAEs resulting in death or a life-threatening situation are reported expeditely.

All AEs are followed until they are resolved, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

Premature termination of the study is considered in case of an unexpected major procedure related SAE that results in death or near-fatal complications. The study team will meet to decide on premature termination of the study in case of frequent (>2) severe complications that, in the opinion of the study team and/or medical monitors, bring into question the safety of the procedure and therewith the safety of the other participants.

Statistical analysis

Statistical analysis will be performed with the Statistical Package for the Social Sciences (version 25.0 for Windows, SPSS Inc., Chicago, USA). For the primary endpoint, the thirty-day procedure related major complication rate will be shown as a percentage by dividing the number of major complications by the number of procedures. Major procedure-related complications are defined as complications that require endovascular or open revascularization, amputation (e.g. treated segment thrombosis, acute onset of limb ischemia), or that might lead to patient death within 30 days of the procedure.

We choose not to perform repeated measurement analyses as we are interested in demonstrating an effect in this first-in-human-use proof-of-concept study, rather than quantifying this effect. Nonetheless, most parameters of the tests and measurements will be represented as continuous variables. The plaque characteristics will be presented as categorical variables and will be analyzed by means of the Fisher's exact test. Changes in these continuous variables will be analyzed by means of the paired T-test or the Mann-Whitney U test, when applicable. Results of analysis will be considered significant when $p < 0.05$. Missing data will be excluded pair-wise.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

ETHICS AND DISSEMINATION

This study is conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013)[32] and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). An Investigator Site File is produced in advance of the study conforming to the institutional guidelines. The study is registered in the Netherlands Trial Register, NTR number NL7564. In case of any protocol amendments needed during the study, the Medical Ethics Committee will be notified. The results of the study are disclosed unreservedly and will be submitted to a peer reviewed scientific journal, in accordance to the CCMO statement[1] containing the basic principles on the disclosure and publication of research results obtained from studies involving human subjects.

DISCUSSION

The HIFU trial is a first-in-human pilot study. The goal of the clinical trial is to investigate the safety and feasibility of robot-assisted high-intensity focused ultrasound using a dual-mode ultrasound array for the treatment of peripheral atherosclerosis. The DMUA allows imaging and delivery of therapy with a single transducer, enabling accurate targeting and a closed-loop control to optimize energy delivery. The local (~1 mm) thermal effect generated by focusing the high-intensity US beams is expected to affect the cycle of plaque formation as it causes coagulation necrosis.[22,33,34] The hypothesis is that the generated heat causes decellularization of the soft plaque segments and reduces the vasa vasorum.[26,35–37] This disrupts the plaque formation, reduces the plaque volume, and increases the vessel lumen diameter. The effect of HIFU therapy on atherosclerosis has only been studied in preclinical studies, and therefore, this first-in-human pilot study is conducted to assess the safety and feasibility of this therapy.

Inclusion and exclusion criteria are set to find eligible candidates for the HIFU therapy. Whether the plaque can be targeted with this specific HIFU is determined from the plaque characteristics, including

[1] https://www.ccmo.nl/onderzoekers/klinisch-onderzoek-naar-medische-hulpmiddelen/tijdens-en-na-onderzoek-naar-medische-hulpmiddelen/resultaten-onderzoek

plaque depth, amount of calcification, and stenosis severity. The depth of the target vessel is dependent on the focus length of the DMUA transducer, which can be set between 45 and 55 mm. Because the transducer cannot be placed directly on the skin due to its concave shape, a water bolus cover is needed to enable good coupling between the skin and transducer. This dictates that the distance between the skin and the HIFU target area cannot exceed 35 mm. The bolus cover can be adjusted to increase the distance between the transducer and skin to allow the target to be in the focal point. Furthermore, altering the pressure in the groin with the transducer can aid in getting the target within therapeutic window.

For the current study, the amount of calcific content in the culprit lesion, arterial depth and stenosis severity are essential for inclusion or exclusion. Severe calcification is considered as a contraindication for the HIFU therapy because the expected therapeutic benefit of this thermal therapy is uncertain.[38] Calcification is an acoustic barrier for US progression and diffusion and can potentially reflect the US beam to non-targeted tissue and therefore highly calcified lesions may not be suitable for HIFU targeting.[38,39] Therefore, for this safety study, highly calcified lesions are contraindicated. Furthermore, in case of a stenosis grade larger than 90%, there may be a risk of occlusion due to clotting and therefore considered as contraindication. The effect of this thermal therapy on atherosclerotic plaque is unknown but in the animal studies some swelling at the targeted vascular wall was observed.[21,40]

Plaque rupture, distal thrombosis, and arterial occlusion are considered as potential complications of this therapy; however, these risks are considered to be low. Preclinical research with this experimental set-up has demonstrated that the endothelium remains intact at the appropriate HIFU intensities, and no rise of temperature in the vessel wall leading to thrombosis occurred.[21] The closed-loop feedback control is also a precaution to prevent overexposure and excessive tissue heating. The endothelial cells of the vessel wall are naturally cooled by the blood flow, thus reducing the chance of plaque rupture.[21] The ongoing trial with this experimental therapy however has to demonstrate that these potential risks do not occur.

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REFERENCES

1. Thukkani AK, Kinlay S, Hospital MB, et al. Endovascular intervention for peripheral artery disease. *Circ Res* 2015; 116: 1599–1613.
2. Bartelink MEL, Elsman BHP, Oostindjer A, et al. NHG-Standaard 'Perifeer arterieel vaatlijden' (tweede herziening). Nederland, 2014.
3. Stoner MC, Calligaro KD, Chaer RA, et al. Reporting standards of the Society for Vascular Surgery for endovascular treatment of chronic lower extremity peripheral artery disease. *Journal of Vascular Surgery* 2016; 64: e1–e21.
4. Nederlandse Vereniging voor Heelkunde & Nederlandse Vereniging voor Radiologie. *Richtlijn: Diagnostiek en behandeling van arterieel vaatlijden van de onderste extremiteit*. 2005.
5. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-society consensus for the management of peripheral arterial disease. *International angiology : a journal of the International Union of Angiology* 2007; 26: 81–157.
6. AbuRahma AF. When Are Endovascular and Open Bypass Treatments Preferred for Femoropopliteal Occlusive Disease? *Annals of Vascular Diseases* 2018; 11: 25–40.
7. Ketteler ER, Brown KR. Radiation exposure in endovascular procedures. *Journal of Vascular Surgery* 2011; 53: 35S-38S.
8. Tang FR, Loganovsky K. Low dose or low dose rate ionizing radiation-induced health effect in the human. *Journal of Environmental Radioactivity* 2018; 192: 32–47.
9. Ko S, Kang S, Ha M, et al. Health Effects from Occupational Radiation Exposure among Fluoroscopy-Guided Interventional Medical Workers: A Systematic Review. *Journal of Vascular and Interventional Radiology* 2018; 29: 353–366.
10. Maďarič J, Mondek P. Comment on 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European society for vascular surgery (ESVS). *Cardiology Letters* 2018; 27: 171–173.
11. Vartanian SM, Conte MS. Surgical Intervention for Peripheral Arterial Disease. *Circulation Research* 2015; 116: 1614–1628.
12. Jia X, Sun ZD, Patel J V., et al. Systematic review of endovascular intervention and surgery for common femoral artery atherosclerotic disease. *British Journal of Surgery* 2019; 106: 13–22.
13. Halpin D, Erben Y, Jayasuriya S, et al. Management of Isolated Atherosclerotic Stenosis of the Common Femoral Artery: A Review of the Literature. *Vascular and Endovascular Surgery* 2017; 51: 220–227.
14. Jaff MR, White CJ, Hiatt WR, et al. An Update on Methods for Revascularization and Expansion of the TASC Lesion Classification to Include Below-the-Knee Arteries: A

Supplement to the Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Vascular Medicine (United Kingdom)* 2015; 20: 465–478.

15. Katsanos K, Spiliopoulos S, Reppas L, et al. Debulking Atherectomy in the Peripheral Arteries: Is There a Role and What is the Evidence? *CardioVascular and Interventional Radiology* 2017; 40: 964–977.
16. Elhelf IAS, Albahar H, Shah U, et al. High intensity focused ultrasound: The fundamentals, clinical applications and research trends. *Diagnostic and Interventional Imaging* 2018; 99: 349–359.
17. ter Haar G. HIFU tissue ablation: Concept and devices. In: *Advances in Experimental Medicine and Biology*. 2016, pp. 3–20.
18. Maloney E, Hwang JH. Emerging HIFU applications in cancer therapy. *International Journal of Hyperthermia* 2015; 31: 302–309.
19. Liu D, Casper A, Haritonova A, et al. Adaptive lesion formation using dual mode ultrasound array system. *AIP Conference Proceedings* 2017; 1821: 1–6.
20. Casper AJ, Liu D, Ballard JR, et al. Real-time implementation of a dual-mode ultrasound array system: In vivo results. *IEEE Transactions on Biomedical Engineering* 2013; 60: 2751–2759.
21. Groen MHA, Slieker FJB, Vink A, et al. Safety and feasibility of arterial wall targeting with robot-assisted high intensity focused ultrasound : a preclinical study. *International Journal of Hyperthermia* 2020; 37: 903–912.
22. Liu D, Ebbini ES, Shehata IA, et al. Feasibility of Targeting Atherosclerotic Plaques by High-Intensity–focused Ultrasound: An In Vivo Study. *Journal of Vascular and Interventional Radiology* 2013; 24: 1880-1887.e2.
23. Aravalli RN, Helden D Van, Liu D, et al. Precision Targeted Ablation of Fine Neurovascular Structures In Vivo Using Dual-mode Ultrasound Arrays. *Scientific Reports* 2020; 10: 9249.
24. Ebbini ES, Ter Haar G. Ultrasound-guided therapeutic focused ultrasound: Current status and future directions. *International Journal of Hyperthermia* 2015; 31: 77–89.
25. Roti Roti JL. Cellular responses to hyperthermia (40-46°C): Cell killing and molecular events. *International Journal of Hyperthermia* 2008; 24: 3–15.
26. Okada M, Hasebe N, Aizawa Y, et al. Thermal Treatment Attenuates Neointimal Thickening with Enhanced Expression of Heat-Shock Protein 72 and Suppression of Oxidative Stress. *Circulation* 2004; 109: 1763–1768.
27. Ebbini ES, Yao H, Shrestha A. Dual-Mode Ultrasound Phased Arrays for Image-Guided Surgery. *Ultrasonic imaging* 2006; 28: 65–82.
28. Patel MR, Conte MS, Cutlip DE, et al. Evaluation and treatment of patients with lower extremity peripheral artery disease: Consensus definitions from peripheral academic

research consortium (PARC). *Journal of the American College of Cardiology* 2015; 65: 931–941.

29. Conte MS. Understanding objective performance goals for critical limb ischemia trials. *Seminars in Vascular Surgery* 2010; 23: 129–137.
30. Goodney PP, Schanzer A, Demartino RR, et al. Validation of the Society for Vascular Surgery's Objective Performance Goals for critical limb ischemia in everyday vascular surgery practice. *Journal of Vascular Surgery* 2011; 54: 100-108.e4.
31. Ng A, Swanevelder J. Resolution in ultrasound imaging. *Continuing Education in Anaesthesia, Critical Care and Pain* 2011; 11: 186–192.
32. World Medical Association. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association* 2013; 310: 2191–2194.
33. Shehata IA, Ballard JR, Casper AJ, et al. Feasibility of targeting atherosclerotic plaques by high-intensity-focused ultrasound: An in vivo study. *Journal of Vascular and Interventional Radiology* 2013; 24: 1880–1887.
34. Palyga I, Palyga R, Mlynarczyk J, et al. The current state and future perspectives of high intensity focused ultrasound (HIFU) ablation for benign thyroid nodules. *Gland Surgery* 2020; 9: S95–S104.
35. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell* 2011; 145: 341–55.
36. Sluimer JC, Gasc JM, van Wanroij JL, et al. Hypoxia, Hypoxia-Inducible Transcription Factor, and Macrophages in Human Atherosclerotic Plaques Are Correlated With Intraplaque Angiogenesis. *Journal of the American College of Cardiology* 2008; 51: 1258–1265.
37. Sedding DG, Boyle EC, Demandt JAF, et al. Vasa vasorum angiogenesis: Key player in the initiation and progression of atherosclerosis and potential target for the treatment of cardiovascular disease. *Frontiers in Immunology*; 9. Epub ahead of print 2018. DOI: 10.3389/fimmu.2018.00706.
38. Copelan A, Hartman J, Chehab M, et al. High-Intensity Focused Ultrasound: Current Status for Image-Guided Therapy. *Seminars in Interventional Radiology* 2015; 32: 398–415.
39. Mearini L, Porena M. Transrectal high-intensity focused ultrasound for the treatment of prostate cancer: past, present, and future. *Indian journal of urology : IJU : journal of the Urological Society of India* 2010; 26: 4–11.
40. Ballard JR, Casper AJ, Liu D, et al. Dual-mode ultrasound arrays for image-guided targeting of atheromatous plaques. *AIP Conference Proceedings* 2012; 1503: 124–128.

Author statement:

All authors declare to have made substantial contributions to the development of this protocol. MS prepared the first draft of the manuscript. MG, RvE, CH, GdB, PD and EE contributed to the writing, editing and revising of the protocol. TL is responsible for patient eligibility.

Figure 1 HIFU/DMUA set-up as used during a clinical procedure

625x308mm (118 x 118 DPI)

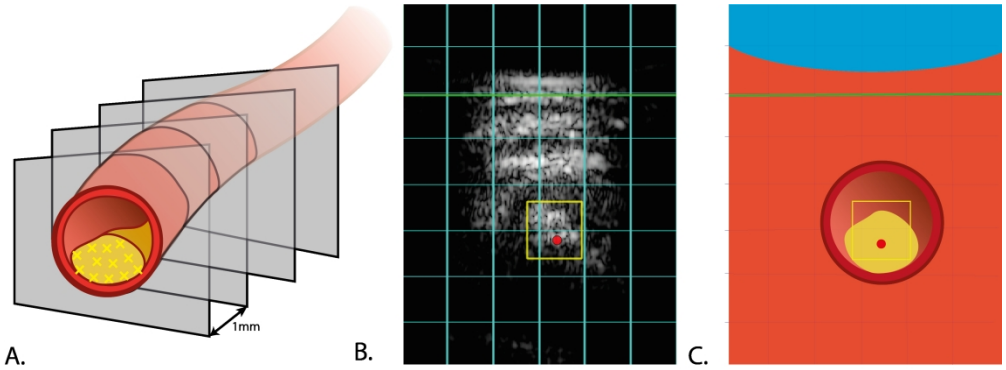

Figure 2 Panel A: Schematic overview of target planes in a vessel. Panel B: Screenshot of the DMUA/HIFU image of the vessel with target area marked by the red dot. The green box represents the skin interface and the yellow box represents the region of interest (ROI). Panel C: schematic overview of the screenshot shown in panel B.

824x302mm (118 x 118 DPI)

Appendix E: Subject Consent Form

First in man use of DMUA/HIFU therapy for the treatment of atherosclerotic plaques in the femoral artery

- I have read the subject information form. I was also able to ask questions. My questions have been answered to my satisfaction. I had enough time to decide whether to participate.
- I know that participation is voluntary. I know that I may decide at any time not to participate after all or to withdraw from the study. I do not need to give a reason for this.
- I give permission for my GP to be informed that I am participating in this study
- I give permission for the collection and use of my data to answer the research question in this study.
- I know that some people may have access to all my data to verify the study. These people are listed in this information sheet. I consent to the inspection by them.
- I agree that my GP and / or treating specialist will be informed of coincidental findings that (may) be of interest for my health.
- I know that I should not become pregnant during the study.
- If applicable: the investigator has discussed with me the most suitable contraception for me.
- I □ **do**
  □ **do not**
  consent to keeping my personal data longer and to use it for future research in the field of my condition and / or the studied treatment method.
- I □ **do**
  □ **do not**
  consent to being contacted again after this study for a follow-up study.
- I □ **do**
  □ **do not**
  want to be informed about what treatment I have received / in which group I was.

- I want to participate in this study.

Name of study subject:

Signature: Date: __ / __ / __

I hereby declare that I have fully informed this study subject about this study.

If information comes to light during the course of the study that could affect the study subject's consent, I will inform him/her of this in a timely fashion.

Name of investigator (or his/her representative):

Signature: Date:__ / __ / __

If applicable

Additional information was given by:

Name:

Job title:

Signature: Date:__ / __ / __

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* Delete as appropriate.

The study subject will receive the full information sheet, together with a signed copy of the consent form.

BMJ Open

Safety and feasibility study of non-invasive robot-assisted high intensity focused ultrasound therapy for the treatment of atherosclerotic plaques in the femoral artery: protocol for a pilot study

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Safety and feasibility study of non-invasive robot-assisted high intensity focused ultrasound therapy for the treatment of atherosclerotic plaques in the femoral artery: protocol for a pilot study

M.V. Simons[1], M.V.Simons-4@umcutrecht.nl, M.H.A. Groen[2], M.H.A.Groen-4@umcutrecht.nl, G.J. de Borst[1], G.J.deBorst-2@umcutrecht.nl, T. Leiner[5], leiner.tim@mayo.edu, P.A.F. Doevendans[2, 3, 4], P.Doevendans@umcutrecht.nl, E.S. Ebbini[6], ebbin001@umn.edu, F.J.B. Slieker[7], F.J.B.Slieker-3@umcutrecht.nl, R. van Es[2], R.vanEs-2@umcutrecht.nl, C.E.V.B. Hazenberg[1], C.E.V.B.Hazenberg@umcutrecht.nl

[1] Department of Vascular Surgery, University Medical Center Utrecht, Utrecht, the Netherlands.

[2] Department of Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands.

[3] Central Military Hospital, Utrecht, The Netherlands.

[4] Netherlands Heart Institute, Utrecht, The Netherlands.

[5] Department of Radiology, University Medical Center Utrecht, Utrecht, The Netherlands.

[6] Department of Electrical and Computer Engineering, University of Minnesota, Minneapolis, MN, USA

[7] Department of Oral Surgery, University Medical Center Utrecht, Utrecht, The Netherlands.

Corresponding author: M.V. Simons, M.V.Simons-4@umcutrecht.nl, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, telephone: +31 6 39034914

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Trial sponsor: University Medical Center Utrecht, Heidelberglaan 100, 3508 GA Utrecht, The Netherlands, telephone: +31 (0)88 7555555

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ABSTRACT

Introduction: Peripheral arterial disease (PAD) is an atherosclerotic disease leading to stenosis and/or occlusion of the arterial circulation of the lower extremities. The currently available revascularization methods have an acceptable initial success rate, but the long-term patency is limited, while surgical revascularization is associated with a relatively high perioperative risk. This urges the need for development of less invasive and more effective treatment modalities. This protocol article describes a study investigating a new non-invasive technique that uses robot assisted high intensity focused ultrasound (HIFU) to treat atherosclerosis in the femoral artery.

Methods and analysis: A pilot study is currently performed in 15 symptomatic PAD patients with a significant stenosis in the common femoral (CFA) and/or proximal superficial femoral artery (SFA). All patients will be treated with the dual-mode ultrasound array (DMUA) system to deliver imaging-guided high-intensity focused ultrasound (HIFU) to the atherosclerotic plaque. Safety and feasibility are the primary objectives assessed by the technical feasibility of this therapy and the 30-day major complication rate as primary endpoints. Secondary endpoints are angiographic and clinical success and quality of life.

Ethics and dissemination: Ethical approval for this study was obtained in 2019 from the Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands. Data will be presented at national and international conferences and published in a peer-reviewed journal.

Trial registration numbers: Netherlands Trial Register, NL7564.

Keywords: Atherosclerosis, focused ultrasound, peripheral arterial disease, plaque debulking, thermal therapy

Strengths and limitations of this study

- First in human study to assess the safety and feasibility of noninvasive HIFU/DMUA therapy to target atherosclerotic plaques in the femoral artery.
- Extensive follow-up in the first month after the procedure with duplex ultrasound and magnetic resonance imaging providing detailed information about the plaque response to the therapy.
- Next to quantitative plaque analysis, patients are asked to fill in quality of life questionnaires specially designed for Dutch patients with peripheral arterial disease.
- This is a non-randomized single center study and consequently there is chance of selection bias.
- Calcified plaques are an exclusion parameter so the data collected in this study cannot be extrapolated to the full spectrum of plaque morphologies.

INTRODUCTION

Peripheral arterial disease (PAD) is an atherosclerotic disease leading to stenosis and/or occlusion of the arterial circulation of the lower extremities. The severity of PAD varies from asymptomatic stenosis to limb-threatening ischemia.[1–3] In case of severe symptoms, revascularization is indicated. Three main options are generally available for revascularization, namely, open, endovascular or a hybrid intervention. The open option is the surgical removal of the atherosclerotic plaque (endarterectomy), or bypass surgery, where a new conduit of blood flow is created to overcome the stenosis or occlusion. The endovascular option is a minimally invasive manner of increasing the lumen diameter by inflating a balloon (angioplasty) combined with expanding a metal scaffold (stent), if indicated. In a hybrid case, open surgery and endovascular surgery are combined. The predicted benefit for patients should outweigh the potential risks and durability of the intervention.[4–6] Most peripheral arterial interventions have an acceptable initial technical and clinical success rate, but the long-term patency is limited.[5] Furthermore, fluoroscopy exposure during these procedures also possesses potential health risks, such as DNA damage and long-term health effects, to both the patient and the treatment team.[7–9]

Guidelines including The Trans-Atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC) generally recommend for revascularization options (open, endovascular or hybrid procedures) based on the anatomical location and extent of the arterial lesions. [4,5,10] Endovascular revascularization is generally preferred over open procedures due to the reduced perioperative morbidity and shorter hospital stays.[1,11] However, endovascular strategies for stenotic lesions located in anatomically ‘‘hostile’’ arterial segments or ‘‘no stent areas,’’ such as arterial flexion points (surrounding joints), remain a subject for debate. For example, the endovascular approach has not been adopted widely in the common femoral artery (groin). Despite the reduction of wound related complications and shorter hospital stay, endovascular revascularization in the common femoral artery is associated with a lower patency and increased rates of subsequent revascularization procedures. Another concern is that stent placement in this region may limit access options for future procedures, and the bending forces may cause stent fracture and subsequent arterial occlusion.[12] For this reason, despite the risk of infection and septic bleeding, open endarterectomy of the common femoral artery is still considered the gold standard therapy.[13,14]

An alternative to the increased complication risk of the open surgical approach is endovascular plaque debulking. With plaque debulking, the plaque is removed and reduced rather than cracked and pressed against the arterial wall, which might lessen vessel barotrauma and reduce the risk of plaque recoil. Directional, rotational, orbital, and laser atherectomy are current examples of endovascular plaque debulking with good technical success rates.[15] Nevertheless, the reported restenosis and subsequent reinterventions rates are comparable to regular endovascular revascularization.[15]

Noninvasive plaque debulking techniques may overcome the risk of perioperative complications associated with surgery altogether. High-intensity focused ultrasound (HIFU) enables noninvasive tissue ablation.[16–18] Dual-mode ultrasound arrays (DMUA) offers the possibility of simultaneous HIFU targeting and ultrasound (US) imaging, thereby allowing inherent registration between imaging and treatment location.[19–21] HIFU/DMUA therapy can be used for transcutaneous noninvasive ablation of targets with submillimeter precision and accuracy.[17,19–24] The pathophysiology of atherosclerosis suggests that atherosclerotic plaques may respond to HIFU/DMUA therapy.[21] It is hypothesized that the local thermal effect of HIFU causes coagulation necrosis affecting the plaque and inducing a new immune response with a complex cascade of cellular reactions, eventually leading to a decrease in plaque volume and an increased lumen diameter.[25,26]

A clinical trial currently being conducted at the University Medical Center Utrecht (UMCU) is investigating the safety and feasibility of this technique (Netherlands Trial Register trial number NL7564). This article describes the working protocol for the treatment of femoral arterial disease with this specific intervention.

Technical background: HIFU/DMUA synthesizer

The DMUA transducer used in this study is specifically developed for arterial plaque debulking purposes.[20] The 3.5-MHz 64-element concave shaped dual mode array transducer (Imasonic, Voray sur l'Ognon, France) uses the spherical shape for mechanical steering of the US beams (Table 1). The transducer is attached to a six degrees-of-freedom robotic arm (UR3, Universal Robots, Odense, Denmark) to allow precise positioning that enables accurate imaging and treatment on a sub-millimeter scale.

The HIFU/DMUA synthesizer is an integrated medical device that delivers US-guided HIFU therapy (figure 1). The DMUA allows real-time monitoring of lesion formation and can therefore be used as a closed-loop feedback to control energy delivery to optimize treatment. HIFU therapy is based on the fact that US beams are partially converted into thermal energy when travelling through tissue. At low intensities, as with diagnostic US, this phenomenon does not interfere with normal physiology. However, when emitted at high intensities, US beams can produce a significant rise in temperature, leading to coagulation necrosis. With HIFU therapy, the high-intensity US beams are focused on diseased tissue, thereby producing localized submillimeter thermal lesions at the targeted tissue leading to coagulation necrosis. For safety measures, the HIFU/DMUA synthesizer is equipped with a closed-loop prescriptive image-guided control (CLC) algorithm. The energy delivery is monitored along the acoustic path of the beam to avoid affecting non-targeted tissues and to modulate the amplitude of the HIFU pulse based on image feedback. This feedback consists of single transmit focus (STF) imaging data that are gathered before and after every 10 milliseconds during the HIFU bursts.[20] STF imaging is a method of high-speed imaging enabling monitoring of the changes at the treatment site.[20,27]

Table 1 DMUA technical specifications

Type	Linear array transducer
Number of channels	2 rows of 32 elements
Mechanical focusing	Spherical
Radius of curvature	50 mm ±2 mm
Inter-element spacing	0.2 mm
Height of the elements	34 mm divided into two rows (equal height)
Frequency	3.5 MHz

Figure 1 HIFU/DMUA set-up as used during a clinical procedure

METHODS AND ANALYSIS

Study objectives

To investigate the safety and feasibility of HIFU/DMUA therapy for the treatment of symptomatic patients with atherosclerotic plaques in the femoral artery.

Study design

The study is designed as a first-in-human pilot study that is currently being conducted by the University Medical Center Utrecht with a cohort of 15 patients diagnosed with PAD. The protocol was finalized February 25, 2019 and first inclusion was performed June 17, 2019. Since then, minor changes to the protocol were made and approved by the Medical Research Ethics Committee (version 1.4). The estimated end date of the study is halfway through 2023, since there has been a significant delay due to the COVID-19 pandemic. All patients are asked to provide written informed consent. After informed consent is signed, patients will undergo a thorough screening to assess eligibility for inclusion.

Primary study parameters

The main endpoints are the safety and feasibility of this experimental treatment. Safety is determined by the 30-day major complication rate. This is a composite safety endpoint, based on previously validated safety endpoints for symptomatic peripheral arterial disease.[28–30] It consists of the 30-day major adverse event rate, which is defined as any complication that requires endovascular revascularization, open revascularization, or amputation in the target limb. Secondly, it consists of the 30-day mortality rate. Feasibility is defined as being able to accomplish the HIFU/DMUA procedure to target the atherosclerotic plaque in the CFA and/or SFA.

Secondary study parameters

Secondary endpoints are defined as technical, vascular imaging, clinical, and quality of life parameters. Technical success is defined as successful visualization of the target lesion and successful delivery of HIFU therapy to the intended target lesions. During the procedure, procedural data of the device, the number, intensity, and duration of all HIFU shots delivered will be recorded and analyzed. The intensity of the shots is depending on the HIFU amplitude and tissue attenuation and absorption but is generally

around 6.25 kW/cm^2.[21] After the HIFU procedure the number of shots and the efficacy of the shots will be determined. The efficacy will be analyzed based on the echogenicity change during the shot. Shots reaching threshold will be considered as effective HIFU shots. Another important aspect of the technical success is the non-occurrence of complications such as thrombosis, dissection or occlusion within the target vessel due to the HIFU therapy.

Follow up by vascular imaging is performed with both MRI and duplex ultrasound (DUS) to visualize plaque morphology and stenosis severity over time. With MRI changes in lesion length (mm), stenosis severity (%), total plaque volume (mm^3), total vascular wall volume (mm^3), and atherosclerotic (peri-) plaque characteristics like presence of edema, calcification, intraplaque hemorrhage and presence of a lipid rich necrotic core can be analyzed. DUS imaging will be used to assess the hemodynamic changes by measuring the peak systolic velocity ratio (PSV ratio) at the target lesion. Furthermore, plaque (surface) characteristics will be described and special attention will be given to assess the occurrence of arteriovenous fistula.

Clinical endpoints are assessed on functional exercise and the ankle-brachial index (ABI) measurements. Hemodynamic success is defined as an improvement in the ABI at 30 days post procedure compared to baseline. Functional success is defined as an improvement on exercise testing. An improvement of symptoms is also taken into account.

Patients and eligibility criteria

The present safety and feasibility study is a pilot being conducted by the University Medical Center Utrecht, the Netherlands. To determine whether HIFU treatment is safe in this study, the safety outcome of HIFU-treatment has to be non-inferior to the safety outcome of the common femoral endarterectomy (CFE). The complication rate of a CFE varies between 6 – 26 %[13], with the point of gravity around 8% (1 in 12.5 patients). Therefore, a sample size of 15 patients is chosen for this safety trial for the investigational therapy. The most relevant eligibility criteria are represented in table 2, the full list of eligibility criteria is added as supplemental information. Patients classified as Fontaine class IIB or III with an isolated CFA or proximal SFA, or multilevel femoral disease (CFA and SFA) but no indication to treat distal the SFA (in case of Fonataine IIB), will be considered to be eligible for inclusion. Written

informed consent is obtained during the baseline visit after a reflection period of at least 7 days after the first study information visit.

Table 2 Eligibility criteria

Inclusion criteria	Exclusion criteria
Patient < 85 years	Patient is diagnosed with early onset PAD
Patient is diagnosed with symptomatic PAD (ABI <0.90)	Contraindication for MRI or gadolinium contrast agent
Primary non-stented target plaque with focal stenosis in CFA/proximal SFA	Recent cardiovascular event or major surgery (<6 months ago)
Presence of lower extremity CTA imaging to measure: - Grade of stenosis 50-90% - Plaque length ≤40mm - Grade of calcification ≤50% - Distance dorsal vessel wall to the skin ≤35 mm	Contra-indication for antiplatelet therapy
Target vessel visible with DMUA/HIFU synthesizer imaging	

Data handling

Participant UMCU healthcare data, data from medical interviews, quality of life questionnaires and data from various medical tests and measurements are filed as source data in the electronic patient records. All study-related results from medical interviews, tests and measurements are collected in an electronic Case Report Form (eCRF) with audit trial functionality. Internal auditing is performed and, independent from the sponsor, monitoring is performed by the Julius Clinical Research (Zeist, The Netherlands). The monitor will visit periodically to discuss progress of the clinical trial, review correspondence and review the CRFs and the original documents with the study personnel for accuracy of data recording. Research data is stored anonymized and personal data is stored separately. A separate file is created to identify

the patient via their unique study number. Research data will be archived for 15 years after the study has ended. To be able to reproduce the study findings, and to help future users understand and reuse data, all changes made to the raw data will be documented in data queries generated in the eCRF.

Study procedures

Screening procedure

The vascular surgeon identifies potential eligible study participants. If the patient consents and meets eligibility criteria as mentioned in table 2 the HIFU specific plaque characteristics (arterial depth, grade of calcification and stenosis severity) will be evaluated in collaboration with a designated expert radiologist. Based on lower extremity computed tomography angiography (CTA) and DUS the calcification grade of maximal 50% in the culprit lesion and arterial depth of maximal 35 mm will be measured (table 2).

Baseline visit

During the baseline visit, patients are asked to sign the informed consent form. After this a clinical interview, physical examination, DUS and magnetic resonance imaging, exercise test and a HIFU/DMUA visibility test is performed. The results of the baseline visit determine if a patient is definitively eligible for study participation.

Intervention

The patient is lying in supine position under sedation during the HIFU procedure. DUS is used initially to visualize and mark the target area on the skin before the HIFU procedure. Anatomical landmarks, such as the CFA/SFA bifurcation, proximal and distal end of the plaque, and calcifications, are marked on the skin as additional guidance for the treatment team during imaging with the DMUA transducer. After this the DMUA transducer is placed in the groin region from where the CFA/SFA plaque can be followed from proximal to distal and eventually targeted. Compared to conventional DUS, image resolution of the DMUA transducer is inferior (axial resolution is ~2.6 mm and lateral resolution is ~1.2 mm whilst conventional DUS can reach submillimeter resolution depending imaging settings)[27,31]. For this reason, additional DUS is required in this phase to ensure correct targeting.

The transducer is attached to a six-degrees-of-freedom robotic arm to ensure precise positioning and accurate imaging of the target area. The transducer is placed in the groin, perpendicular to the CFA to create a transversal plane through the artery. When the CFA is visualized, the robot arm is used to move the transducer along the vessel pathway to visualize the CFA bifurcation and proximal part of the SFA. During this visualization step, synthetic aperture (SA) imaging[27] is used for real-time visualization of the full trajectory. When the full trajectory is visualized and the target planes are determined, the HIFU therapy can start. Per transversal plane (figure 2) multiple shots will be delivered in the stenosis area with an inter-target distance of 1 mm. When a full plane is targeted, the robot moves the transducer 1mm to repeat the process of targeting in the next transversal plane (figure 2). This process is repeated for 10 planes and after this the transducer is moved up to visually inspect the skin region for eventual thermal damage. When there is no sign of thermal damage the transducer can be moved back to the position and the therapy will be continued.

Figure 2 Panel A: Schematic overview of target planes in a vessel. Panel B: Screenshot of the DMUA/HIFU image of the vessel with target area marked by the red dot. The green box represents the skin interface and the yellow box represents the region of interest (ROI). Panel C: schematic overview of the screenshot shown in panel B.

When the stenosis is treated in full length, the skin is again inspected for thermal damage and the lower extremity is assessed for any signs of distal thromboembolism or arterial occlusion. The vascular surgeon also checks for acute vascular patency with DUS.

Follow-up

The overall duration of the follow-up for study participants is three months at specific moments in time. Full follow-up scheme with associated tests and measurements is visible in table 3. During the follow-up patients are assessed on change in symptoms and eventual changes in the stenosis severity based on different imaging modalities (DUS, MRA). Hemodynamic changes are assessed based on the ABI and exercise test.

	Baseline	**Procedure day**	**+1 day**	**+7 days**	**+14 days**	**+21 days**	**+30 days**	**+90 days**
Standardized Medical interview	+	+	+	+	+	+	+	+
Physical examination	+	+	+	+	+	-	+	+

Ankle brachial index	+	+	+	+	+	-	+	+
MRA	+	-	+		-	-	+	-
DUS	+	+	+	+	+	-	+	+
Exercise test	+	-	-	-	-	-	+	-
*Quality of life questionnaire**	+	-	+	-	+	-	+	+

Table 3 Follow-up scheme for study participants. The + sign indicates the test is performed during the visit.
**Standardized and validated vascular quality of life questionnaires are used; VascuQoL-NL/EQ-5D-3L*[32]

Monitoring

Data safety monitoring will be performed by the research team and two independent experts (both UMCU radiologists with experience in the field of magnetic resonance guided HIFU therapy), who shall function as medical safety monitors. The research team and medical safety monitors will monitor any adverse event to evaluate the safety of the HIFU-treatment.

Adverse events

In case of an adverse event (AE) the research team will convene and discuss this within a week, to evaluate whether the AE was procedure-related and to evaluate the safety of the HIFU-treatment. Two independent experts will periodically (every 5 patients) review all AEs. In case of a serious adverse event (SAE), the independent experts are informed <7 days. In case of life-threatening adverse events or death, the independent experts are informed <3 days.

SAE's are reported through the web portal '*ToetsingOnline*' to the accredited Medical Ethics Committee that approved the protocol and to the *'Inspectie gezondheidszorg en jeugd (IGJ)'*, within 15 days after first knowledge of the SAE. SAEs resulting in death or a life-threatening situation are reported expeditely.

All AEs are followed until they are resolved, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

Premature termination of the study is considered in case of an unexpected major procedure related SAE that results in death or near-fatal complications. The study team will meet to decide on premature termination of the study in case of frequent (>2) severe complications that, in the opinion of the study team and/or medical monitors, bring into question the safety of the procedure and therewith the safety of the other participants.

The sponsor also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23rd June 2003). This assurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

Statistical analysis

Statistical analysis will be performed with the Statistical Package for the Social Sciences (version 25.0 for Windows, SPSS Inc., Chicago, USA). For the primary endpoint, the thirty-day procedure related major complication rate will be shown as a percentage by dividing the number of major complications by the number of procedures. Major procedure-related complications are defined as complications that require endovascular or open revascularization, amputation (e.g. treated segment thrombosis, acute onset of limb ischemia), or that might lead to patient death within 30 days of the procedure.

We choose not to perform repeated measurement analyses as we are interested in demonstrating an effect in this first-in-human-use proof-of-concept study, rather than quantifying this effect. Nonetheless, most parameters of the tests and measurements will be represented as continuous variables. The plaque characteristics will be presented as categorical variables and will be analyzed by means of the Fisher's exact test. Changes in these continuous variables will be analyzed by means of the paired T-test or the Mann-Whitney U test, when applicable. Results of analysis will be considered significant when $p < 0.05$. Missing data will be excluded pair-wise.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research. Participants are asked to give feedback on their experience during the conduct of the study. The participants will be informed once the trial results are published.

ETHICS AND DISSEMINATION

This study is conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013)[33] and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). The study design was approved by the Medical Research Ethics

Committee of University Medical Center Utrecht, The Netherlands. An Investigator Site File is produced in advance of the study conforming to the institutional guidelines. The study is registered in the Netherlands Trial Register, NTR number NL7564. In case of any protocol amendments needed during the study, the Medical Ethics Committee will be notified. The results of the study are disclosed unreservedly and will be submitted to a peer reviewed scientific journal, in accordance to the CCMO statement[1] containing the basic principles on the disclosure and publication of research results obtained from studies involving human subjects.

DISCUSSION

The HIFU trial is a first-in-human pilot study. The goal of the clinical trial is to investigate the safety and feasibility of robot-assisted high-intensity focused ultrasound using a dual-mode ultrasound array for the treatment of peripheral atherosclerosis. The DMUA allows imaging and delivery of therapy with a single transducer, enabling accurate targeting and a closed-loop control to optimize energy delivery. The local (~1 mm) thermal effect generated by focusing the high-intensity US beams is expected to affect the cycle of plaque formation as it causes coagulation necrosis.[22,34,35] The hypothesis is that the generated heat causes decellularization of the soft plaque segments and reduces the vasa vasorum.[26,36–38] This disrupts the plaque formation, reduces the plaque volume, and increases the vessel lumen diameter. The effect of HIFU therapy on atherosclerosis has only been studied in preclinical studies, and therefore, this first-in-human pilot study is conducted to assess the safety and feasibility of this therapy.

Inclusion and exclusion criteria are set to find eligible candidates for the HIFU therapy. Whether the plaque can be targeted with this specific HIFU is determined from the plaque characteristics, including plaque depth, amount of calcification, and stenosis severity. The depth of the target vessel is dependent on the focus length of the DMUA transducer, which can be set between 45 and 55 mm. Because the transducer cannot be placed directly on the skin due to its concave shape, a water bolus cover is needed to enable good coupling between the skin and transducer. This dictates that the distance between the skin and the HIFU target area cannot exceed 35 mm. The bolus cover can be adjusted to increase the distance between the transducer and skin to allow the target to be in the focal point. Furthermore,

[1] https://www.ccmo.nl/onderzoekers/klinisch-onderzoek-naar-medische-hulpmiddelen/tijdens-en-na-onderzoek-naar-medische-hulpmiddelen/resultaten-onderzoek

altering the pressure in the groin with the transducer can aid in getting the target within therapeutic window.

For the current study, the amount of calcific content in the culprit lesion, arterial depth and stenosis severity are essential for inclusion or exclusion. Severe calcification is considered as a contraindication for the HIFU therapy because the expected therapeutic benefit of this thermal therapy is uncertain.[39] Calcification is an acoustic barrier for US progression and diffusion and can potentially reflect the US beam to non-targeted tissue and therefore highly calcified lesions may not be suitable for HIFU targeting.[39,40] Therefore, for this safety study, highly calcified lesions are contraindicated. Furthermore, in case of a stenosis grade larger than 90%, there may be a risk of occlusion due to clotting and therefore considered as contraindication. The effect of this thermal therapy on atherosclerotic plaque is unknown but in the animal studies some swelling at the targeted vascular wall was observed.[21,41]

Plaque rupture, distal thrombosis, and arterial occlusion are considered as potential complications of this therapy; however, these risks are considered to be low. Preclinical research with this experimental set-up has demonstrated that the endothelium remains intact at the appropriate HIFU intensities, and no rise of temperature in the vessel wall leading to thrombosis occurred.[21] The closed-loop feedback control is also a precaution to prevent overexposure and excessive tissue heating. The endothelial cells of the vessel wall are naturally cooled by the blood flow, thus reducing the chance of plaque rupture.[21] The ongoing trial with this experimental therapy however has to demonstrate that these potential risks do not occur.

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REFERENCES

1. Thukkani AK, Kinlay S, Hospital MB, et al. Endovascular intervention for peripheral artery disease. *Circ Res* 2015; 116: 1599–1613.
2. Bartelink MEL, Elsman BHP, Oostindjer A, et al. NHG-Standaard 'Perifeer arterieel vaatlijden' (tweede herziening). Nederland, 2014.
3. Stoner MC, Calligaro KD, Chaer RA, et al. Reporting standards of the Society for Vascular Surgery for endovascular treatment of chronic lower extremity peripheral artery disease. *Journal of Vascular Surgery* 2016; 64: e1–e21.
4. Nederlandse Vereniging voor Heelkunde & Nederlandse Vereniging voor Radiologie. *Richtlijn: Diagnostiek en behandeling van arterieel vaatlijden van de onderste extremiteit*. 2005.
5. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-society consensus for the management of peripheral arterial disease. *International angiology : a journal of the International Union of Angiology* 2007; 26: 81–157.
6. AbuRahma AF. When Are Endovascular and Open Bypass Treatments Preferred for Femoropopliteal Occlusive Disease? *Annals of Vascular Diseases* 2018; 11: 25–40.
7. Ketteler ER, Brown KR. Radiation exposure in endovascular procedures. *Journal of Vascular Surgery* 2011; 53: 35S-38S.
8. Tang FR, Loganovsky K. Low dose or low dose rate ionizing radiation-induced health effect in the human. *Journal of Environmental Radioactivity* 2018; 192: 32–47.
9. Ko S, Kang S, Ha M, et al. Health Effects from Occupational Radiation Exposure among Fluoroscopy-Guided Interventional Medical Workers: A Systematic Review. *Journal of Vascular and Interventional Radiology* 2018; 29: 353–366.
10. Maďarič J, Mondek P. Comment on 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European society for vascular surgery (ESVS). *Cardiology Letters* 2018; 27: 171–173.
11. Vartanian SM, Conte MS. Surgical Intervention for Peripheral Arterial Disease. *Circulation Research* 2015; 116: 1614–1628.
12. Jia X, Sun ZD, Patel J V., et al. Systematic review of endovascular intervention and surgery for common femoral artery atherosclerotic disease. *British Journal of Surgery* 2019; 106: 13–22.
13. Halpin D, Erben Y, Jayasuriya S, et al. Management of Isolated Atherosclerotic Stenosis of the Common Femoral Artery: A Review of the Literature. *Vascular and Endovascular Surgery* 2017; 51: 220–227.
14. Jaff MR, White CJ, Hiatt WR, et al. An Update on Methods for Revascularization and Expansion of the TASC Lesion Classification to Include Below-the-Knee Arteries: A

Supplement to the Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Vascular Medicine (United Kingdom)* 2015; 20: 465–478.

15. Katsanos K, Spiliopoulos S, Reppas L, et al. Debulking Atherectomy in the Peripheral Arteries: Is There a Role and What is the Evidence? *CardioVascular and Interventional Radiology* 2017; 40: 964–977.
16. Elhelf IAS, Albahar H, Shah U, et al. High intensity focused ultrasound: The fundamentals, clinical applications and research trends. *Diagnostic and Interventional Imaging* 2018; 99: 349–359.
17. ter Haar G. HIFU tissue ablation: Concept and devices. In: *Advances in Experimental Medicine and Biology*. 2016, pp. 3–20.
18. Maloney E, Hwang JH. Emerging HIFU applications in cancer therapy. *International Journal of Hyperthermia* 2015; 31: 302–309.
19. Liu D, Casper A, Haritonova A, et al. Adaptive lesion formation using dual mode ultrasound array system. *AIP Conference Proceedings* 2017; 1821: 1–6.
20. Casper AJ, Liu D, Ballard JR, et al. Real-time implementation of a dual-mode ultrasound array system: In vivo results. *IEEE Transactions on Biomedical Engineering* 2013; 60: 2751–2759.
21. Groen MHA, Slieker FJB, Vink A, et al. Safety and feasibility of arterial wall targeting with robot-assisted high intensity focused ultrasound : a preclinical study. *International Journal of Hyperthermia* 2020; 37: 903–912.
22. Liu D, Ebbini ES, Shehata IA, et al. Feasibility of Targeting Atherosclerotic Plaques by High-Intensity–focused Ultrasound: An In Vivo Study. *Journal of Vascular and Interventional Radiology* 2013; 24: 1880-1887.e2.
23. Aravalli RN, Helden D Van, Liu D, et al. Precision Targeted Ablation of Fine Neurovascular Structures In Vivo Using Dual-mode Ultrasound Arrays. *Scientific Reports* 2020; 10: 9249.
24. Ebbini ES, Ter Haar G. Ultrasound-guided therapeutic focused ultrasound: Current status and future directions. *International Journal of Hyperthermia* 2015; 31: 77–89.
25. Roti Roti JL. Cellular responses to hyperthermia (40-46°C): Cell killing and molecular events. *International Journal of Hyperthermia* 2008; 24: 3–15.
26. Okada M, Hasebe N, Aizawa Y, et al. Thermal Treatment Attenuates Neointimal Thickening with Enhanced Expression of Heat-Shock Protein 72 and Suppression of Oxidative Stress. *Circulation* 2004; 109: 1763–1768.
27. Ebbini ES, Yao H, Shrestha A. Dual-Mode Ultrasound Phased Arrays for Image-Guided Surgery. *Ultrasonic imaging* 2006; 28: 65–82.
28. Patel MR, Conte MS, Cutlip DE, et al. Evaluation and treatment of patients with lower extremity peripheral artery disease: Consensus definitions from peripheral academic

research consortium (PARC). *Journal of the American College of Cardiology* 2015; 65: 931–941.

29. Conte MS. Understanding objective performance goals for critical limb ischemia trials. *Seminars in Vascular Surgery* 2010; 23: 129–137.
30. Goodney PP, Schanzer A, Demartino RR, et al. Validation of the Society for Vascular Surgery's Objective Performance Goals for critical limb ischemia in everyday vascular surgery practice. *Journal of Vascular Surgery* 2011; 54: 100-108.e4.
31. Ng A, Swanevelder J. Resolution in ultrasound imaging. *Continuing Education in Anaesthesia, Critical Care and Pain* 2011; 11: 186–192.
32. Leersum NJ van, Knaap ETW van der. Validatie Vascular Quality Of Life Questionnaire-6-NL (VASCUQOL-6-NL). *Dutch Institute For CLinical Auditing*; 18.
33. World Medical Association. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association* 2013; 310: 2191–2194.
34. Shehata IA, Ballard JR, Casper AJ, et al. Feasibility of targeting atherosclerotic plaques by high-intensity-focused ultrasound: An in vivo study. *Journal of Vascular and Interventional Radiology* 2013; 24: 1880–1887.
35. Palyga I, Palyga R, Mlynarczyk J, et al. The current state and future perspectives of high intensity focused ultrasound (HIFU) ablation for benign thyroid nodules. *Gland Surgery* 2020; 9: S95–S104.
36. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell* 2011; 145: 341–55.
37. Sluimer JC, Gasc JM, van Wanroij JL, et al. Hypoxia, Hypoxia-Inducible Transcription Factor, and Macrophages in Human Atherosclerotic Plaques Are Correlated With Intraplaque Angiogenesis. *Journal of the American College of Cardiology* 2008; 51: 1258–1265.
38. Sedding DG, Boyle EC, Demandt JAF, et al. Vasa vasorum angiogenesis: Key player in the initiation and progression of atherosclerosis and potential target for the treatment of cardiovascular disease. *Frontiers in Immunology*; 9. Epub ahead of print 2018. DOI: 10.3389/fimmu.2018.00706.
39. Copelan A, Hartman J, Chehab M, et al. High-Intensity Focused Ultrasound: Current Status for Image-Guided Therapy. *Seminars in Interventional Radiology* 2015; 32: 398–415.
40. Mearini L, Porena M. Transrectal high-intensity focused ultrasound for the treatment of prostate cancer: past, present, and future. *Indian journal of urology : IJU : journal of the Urological Society of India* 2010; 26: 4–11.
41. Ballard JR, Casper AJ, Liu D, et al. Dual-mode ultrasound arrays for image-guided

targeting of atheromatous plaques. *AIP Conference Proceedings* 2012; 1503: 124–128.

Author statement:

All authors declare to have made substantial contributions to the development of this protocol. MS prepared the first draft of the manuscript. MG, RvE, CH, GdB, PD and EE contributed to the writing, editing and revising of the protocol. FS was involved in the initial protocol drafting and submission to the Medical Ethics Research Committee. TL is responsible for patient eligibility.

Competing interests statement:

Dr. Hazenberg reports grants from International Cardio Corporation during the conduct of the study. He is a consultant for Cook Medical, Gore Medical, and Terumo Aortic. Prof. Doevendans is one of the founders of the International Cardio Corporation; Prof. Ebbini reports grants, personal fees and non-financial support from International Cardio Corporation, during the conduct of the study; grants and personal fees from International Cardio Corporation, grants and personal fees from National Institutes of Health, outside the submitted work; In addition, prof. Ebbini has a patent Dual mode ultrasound transducer (DMUT) system and method for controlling delivery of ultrasound therapy with royalties paid to International Cardio Corporation, a patent Vascular characterization using ultrasound imaging with royalties paid to International Cardio Corporation, and a patent ultrasound image formation and/or reconstruction using multiple frequency waveforms with royalties paid to International Cardio Corporation.; The other authors have nothing to disclose.

Figure 1 HIFU/DMUA set-up as used during a clinical procedure

625x308mm (118 x 118 DPI)

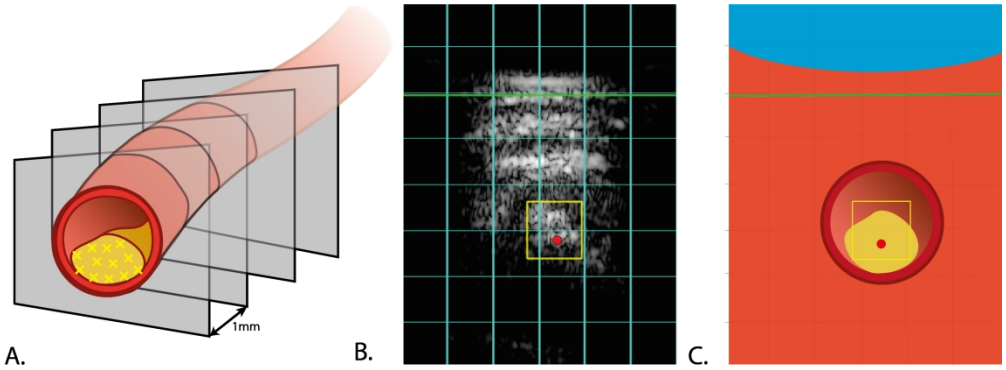

Figure 2 Panel A: Schematic overview of target planes in a vessel. Panel B: Screenshot of the DMUA/HIFU image of the vessel with target area marked by the red dot. The green box represents the skin interface and the yellow box represents the region of interest (ROI). Panel C: schematic overview of the screenshot shown in panel B.

824x302mm (118 x 118 DPI)

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Maximal age of the patient is 85 years.
2. Individual is diagnosed with symptomatic peripheral arterial disease (ankle brachial index <0,9), with focal localisation in the femoral artery.
3. Individual has a non-stented, primary target lesion with a 50-90% occlusion or symptoms with a total lesion length of ≤40mm.
4. Presence of CTA-imaging of the target lesion in the patient's medical file at baseline (<2 year old), from which the degree of plaque calcification can be measured.
5. On the CTA imaging an estimation of the arterial depth can be obtained. If the measured distance exceeds 35 mm proposed is to assess available duplex-ultrasound imaging for arterial depth measurement. The pressure of the ultrasound probe used during a duplex is comparable with the generated probe pressure during HIFU therapy.
6. The target vessel and/or lesion must be visible on ultrasound-imaging of the DMUA/HIFU-system.

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Patient is diagnosed with early onset peripheral arterial disease.
2. The maximum distance from the skin surface to the dorsal vessel wall exceeds 35 mm based on ultrasound imaging.
3. The research team is unable to locate the target vessel/lesion with ultrasound-imaging of the DMUA/HIFU-system.
4. Volume of calcified areas in the plaque more than 50% of the culprit lesion, and/or distribution of calcification in the culprit lesion which the research team considers not suitable for HIFU-treatment after preprocedural assessment of the existing CTA-imaging.
5. Plaque that in the opinion of the research team is unsuitable for HIFU-treatment after baseline screening of patients with MRA and/or echo-duplex. For example, unstable plaque (e.g. thin fibrous cap, or intraplaque haemorrhage).
6. Presence of any anatomical structures located near the focus of the HIFU beam, which in the opinion of the study team, would interfere with safe delivery of the therapy (e.g. nerves, bone, extensive scar tissue).
7. History of prior femoral artery stenting at the contemplated target location.

8. Recent (<6 months) cardiovascular event (myocardial infarction, unstable angina pectoris, TIA/CVA) or major surgery.
9. Contraindication for antiplatelet therapy.
10. Any serious medical condition or any other (medical, physical, anatomical) considerations, which in the opinion of the study team may adversely affect the safety of the participant in the study.
11. Individual has any contraindications for any of the study investigations (e.g. claustrophobia for MRI).
12. Individual has a known, unresolved history of substance abuse or alcohol dependency, lacks the ability to comprehend or follow instructions or would be unlikely or unable to comply with the study protocol.
13. Individual is currently enrolled in another investigational or device trial.
14. Individual is pregnant, nursing or planning to be pregnant.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	#3	Date and version identifier	8
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	2
Roles and	#5b	Name and contact information for the trial sponsor	17

responsibilities: sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	na
Objectives	#7	Specific objectives or hypotheses	6&8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	na
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	na
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	na
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods:			
Assignment of			

interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	na
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	na
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	na
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	na
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	na
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10

Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	14

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Supplement, informed consent form
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	2
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	supplement

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	na