WHAT THIS PAPER ADDS TO THE EXISTING LITERATURE AND FUTURE CLINICAL PRACTICE

This study reports the first in man study to examine the safety and technical feasibility of therapeutic microbubbles, combined with the application of ultrasound and catheter-directed thrombolysis in peripheral arterial occlusions. A safe and technically feasible application of this new technique will result in the set-up of a multicenter randomized clinical trial to compare the efficacy and outcomes with conventional thrombolytic treatment.
ABSTRACT

INTRODUCTION
Acute peripheral arterial occlusions can be treated with intra-arterial catheter-directed thrombolysis as an alternative to surgical thromboembolectomy. Although less invasive, this treatment is time-consuming and carries a significant risk of hemorrhagic complications. Contrast-enhanced ultrasound using microbubbles could accelerate dissolution of thrombi by thrombolytic medications due to mechanical effects caused by oscillation; this could allow for lower dosages of thrombolytics and faster thrombolysis, thereby reducing the risk of hemorrhagic complications. In this study, the safety and practical applicability of this treatment will be investigated.

METHODS AND ANALYSIS
A single-arm phase-II trial will be performed in 20 patients with acute peripheral arterial occlusions eligible for thrombolytic treatment. Low-dose catheter-directed thrombolysis with urokinase will be used. The investigated treatment will be performed during the first hour of thrombolysis, consisting of intravenous infusion of 4 Luminity vials (1.5 mL each, diluted with saline 0.9% to 40 mL total) of microbubbles with the use of local ultrasound at the site of occlusion. Primary endpoints are the incidence of complications and technical feasibility. Secondary endpoints are angiographic and clinical success, duration of thrombolytic infusion, treatment-related mortality, amputations, additional interventions, and quality of life.

ETHICS AND DISSEMINATION
Ethical approval for this study was obtained in 2015 from the Medical Ethics Committee (METC) of the VU University Medical Center, Amsterdam, the Netherlands. A statement of consent for this study was given by the Dutch national competent authority. Data will be presented at national and international conferences and published in a peer-reviewed journal.

REGISTRATION
Dutch National Trial Registry: NTR4731; European Clinical Trials Database (EudraCT) of the European Medicines Agency: 2014-003469-10.

STRENGTHS AND LIMITATIONS
- This will be a first in man study to examine the safety and technical feasibility of therapeutic microbubbles, combined with the application of ultrasound and catheter-directed thrombolysis in peripheral arterial occlusions.
- This is a ‘single arm’ trial. The data will be used to inform a future large multicentre randomised controlled trial comparing conventional catheter-directed thrombolysis with microbubble and ultrasound accelerated thrombolysis.
- The present study is a non-randomized Phase II-trial, therefore the results cannot confirm benefit of sono-thrombolysis for peripheral arterial occlusions, only safety and feasibility is analyzed.
- The present study does not compare other thrombolysis techniques or protocols.
INTRODUCTION

Acute limb ischemia can be caused by a thrombus occluding an artery in an arm or leg. This is an emergency situation that can result in amputation or death if not treated successfully.\(^1\) Intra-arterial infusion of thrombolytic agents, i.e. catheter-directed thrombolysis, can restore blood flow by dissolving the clot, as a less invasive alternative to surgical thromboembolectomy.\(^2\) In comparison with the lysis of small arterial occlusions in patients with myocardial infarction, larger peripheral arterial occlusions require higher doses of lytic agents and infusion over a longer period of time. Inevitably, such treatment is accompanied by a risk of major hemorrhagic complications, such as hemorrhagic stroke, in up to 8% of patients.\(^3\) Furthermore, this technique is time-consuming (several days of bed rest is usually required) and repeated angiography for treatment monitoring is needed, putting patients at risk for contrast-induced nephropathy. As a result, this leads to high morbidity rates and a significant patient burden. Methods to improve this therapy are therefore highly sought after.

A potential accelerator of thrombolytic therapy is contrast-enhanced ultrasound. Contrast agents, consisting of 5-10 µm gas-filled particles (microbubbles), have initially been used as diagnostic ultrasound contrast-enhancers. A new field of research investigates these agents for potential therapeutic purposes such as targeted drug delivery and thrombolysis.\(^4\) The proposed mechanism of action in thrombolysis is that microbubbles can oscillate under the influence of ultrasound. At high intensities, this oscillation can lead to microbubble collapse and the production of mechanical forces on the clot surface, making the thrombus more susceptible to thrombolytics, thus accelerating thrombolysis.\(^5\)

In early stages of clinical research, this technique has been shown to be efficient as treatment for acute cerebral stroke and acute myocardial.\(^6,7\) Although the safety of their clinical administration in treating smaller arteries in the heart has been a topic of discussion in the past, post-marketing data for diagnostic indications showed continued safety after extensive research in more recent years.\(^8,9,10\) For therapeutic thrombolytic purposes, this technique has been shown to be effective and safe in a porcine model of large peripheral arterial occlusions.\(^11\) In this study, we will investigate the therapeutic application of microbubbles with ultrasound in combination with catheter-directed thrombolysis for patients with peripheral arterial occlusions. An illustrative video regarding our research project is available as supplementary video.
CHAPTER 7

METHODS AND ANALYSIS

STUDY OBJECTIVES

To investigate the safety and practical applicability of the therapeutic application of microbubbles and ultrasound in combination with catheter-directed thrombolysis for patients with peripheral arterial occlusions.

DESIGN

The Microbubbles and UltraSound accelerated Thrombolysis (MUST) trial is a single-arm phase-II trial.

PRIMARY STUDY PARAMETERS

Main endpoints will be the safety and technical feasibility of the experimental treatment. Safety will be determined by treatment-related mortality, the occurrence of Adverse Events (AEs), Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs). AEs will be defined as any undesirable experience occurring to a subject during the experimental treatment period, whether or not considered to be related to the investigational drug or intervention. SAEs will be defined as any untoward medical occurrence or effect that at any dose results in death; is life threatening (at the time of the event); requires hospitalization or prolongation of existing in-patients' hospitalization; results in persistent or significant disability or incapacity; is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction. SUSARs, which are related to the microbubble infusion and ultrasound application, are the formation of microembolisms resulting in occlusion of the microcirculation, hemorrhages, hypotension, heart rhythm disorders and anaphylaxis. See the paragraph adverse events for detailed handling procedures of AEs, SAEs and SUSARs. Hemorrhagic complications related to thrombolytic therapy will be reported according the Standardized Bleeding Definitions for Cardiovascular Clinical Trials proposed by Mehran et al. Technical feasibility will be defined as accomplishment of the experimental protocol during the first hour of thrombolysis.

SECONDARY STUDY PARAMETERS

Angiographic success will be defined as dissolution of >95% of the thrombus with outflow to at least 1 crural artery. Clinical change/success will be reported according to Rutherford’s recommended scale for gauging changes in clinical status (Table 7.1). Amputations will be defined as either major (above or below knee amputation) or minor (metatarsal or toe amputation). Additional interventions will be categorized as either surgical (for example thromboembolectomy, bypass graft surgery) or percutaneous (Percutaneous Transluminal Angioplasty, stent placement) and as either required for restoration of patency or necessary for correction of underlying lesions. We will also determine microcirculation of the limb (by Laser Doppler measurements, Perimed Instruments, Järfälla, Sweden), 30-day mortality, conversion to surgery, serum fibrinogen concentrations measured during thrombolytic treatment on a daily basis, pain by Visual Analogue Scale (VAS) and quality of life by SF-36 questionnaires. The
duration of thrombolysis will be defined by the time-span between initiation and completion angiography.

**TABLE 7.1**
RUTHERFORD’S RECOMMENDED SCALE FOR GAUGING CHANGES IN CLINICAL

<table>
<thead>
<tr>
<th>Change</th>
<th>Description</th>
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<tbody>
<tr>
<td>+3</td>
<td>Markedly improved: No ischemic symptoms, and any foot lesions completely healed; ABI essentially “normalized” (increased to more than 0.90)</td>
</tr>
<tr>
<td>+2</td>
<td>Moderately improved: No open foot lesions; still symptomatic but only with exercise and improved by at least one clinical chronic ischemia category; ABI not normalized but increased by more than 0.10</td>
</tr>
<tr>
<td>+1</td>
<td>Minimally improved: Greater than 0.10 increase in ABI* but no categorical improvement or vice versa (i.e., upward categorical shift without an increase in ABI of more than 0.10)</td>
</tr>
<tr>
<td>0</td>
<td>No change: No categorical shift and less than 0.10 change in ABI</td>
</tr>
<tr>
<td>−1</td>
<td>Mildly worse: No categorical shift but ABI decreased more than 0.10, or downward categorical shift with ABI decrease less than 0.10</td>
</tr>
<tr>
<td>−2</td>
<td>Moderately worse: One category worse or unexpected minor amputation</td>
</tr>
</tbody>
</table>


Abbreviations: ABI=Ankle Brachial Index

*In cases where the ABI cannot be accurately measured, an index based on the toe pressure, or any measurable pressure distal to the site of revascularization, may be substituted.

**PATIENTS AND ELIGIBILITY CRITERIA**

The present feasibility and safety study is a non-randomized Phase II trial, to be conducted in our university hospital in Amsterdam, the Netherlands. Usually in a Phase-II trial, 10–20 patients are investigated to confirm an occurrence of toxic effects or serious adverse events <20%. We chose a sample size of 20 to assess the safety of the investigational treatment. Eligibility criteria are listed in Table 7.2. Inclusion of 20 eligible patient is expected within 1.5 years. Written informed consent will be acquired by a member of the Research Team after information about the study has been provided by the treating doctor.

**DATA HANDLING**

We will keep an electronic log of patients who fulfill the eligibility criteria, patients who are invited to participate in the study, patients recruited and patients who withdraw from the study. Reasons for non-recruitment will also be recorded. We will attempt to collect reasons
for non-participation from patients who decline to take part. During the course of the study, we will document reasons for withdrawal from the study and loss to follow-up. Data will be stored electronically in Case Report Forms software with audit trail functionality and will be audited by the institutional Clinical Research Bureau (CRB). Only anonymized information will be stored and participants will only be identifiable by their unique study number, which will be kept in a separate file. Data will be securely stored on these servers for 15 years according to national guidelines. The principal investigator will have access to the final trial dataset. No independent Data Management Committee was instated according to local ethics committee guidelines since the present study was not classified as a high-risk clinical study. This classification was approved by the local ethics committee based on the risk assessment form of the Netherlands Federation of University Medical Centres.

TABLE 7.2
ELIGIBILITY CRITERIA

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Men and women older than 18 and younger than 85 years old</td>
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<tr>
<td>Patients with a maximum of 2 weeks of symptoms for lower limb ischemia due to thrombosed/occluded iliofemoral, femoropopliteal or femorocrural native arteries or iliofemoral, femoropopliteal or femorocrural venous or prosthetic bypass grafts</td>
</tr>
<tr>
<td>Patients appropriate for thrombolysis i.e. with acute lower limb ischemia class I and IIa according to the Rutherford classification</td>
</tr>
<tr>
<td>Patients who understand the nature of the procedure and provide written informed consent before enrollment in the study</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with clinical complaints of acute lower limb ischemia due to thrombosis of iliofemoral, femoropopliteal or femorocrural native arteries, or iliofemoral, femoropopliteal or femorocrural venous or prosthetic bypass grafts for more than 2 weeks</td>
</tr>
<tr>
<td>Patients with thrombosed popliteal aneurysms</td>
</tr>
<tr>
<td>Patients with absolute contraindications for administration of antiplatelet therapy, anticoagulants or thrombolytics</td>
</tr>
<tr>
<td>History of recent (less than 6 weeks) ischemic stroke, cerebral hemorrhagic or myocardial infarction</td>
</tr>
<tr>
<td>Patients with recent (less than 6 weeks) surgery</td>
</tr>
<tr>
<td>Severe hypertension (diastolic blood pressure greater than 110 mm Hg, systolic blood pressure higher than 200 mm Hg)</td>
</tr>
<tr>
<td>Current malignancy or severe co-morbid condition with a life expectancy of less than 6 months</td>
</tr>
<tr>
<td>Patients with uncorrected bleeding disorders (gastrointestinal ulcer, menorrhagia, liver failure)</td>
</tr>
<tr>
<td>Women with childbearing potential not taking adequate contraceptives or currently breastfeeding</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>Patients who are currently participating in another investigational drug or device study</td>
</tr>
<tr>
<td>Patients younger than 18 years or older than 85 years</td>
</tr>
<tr>
<td>Patients with contraindications for Luminity microbubbles i.e.:</td>
</tr>
<tr>
<td>Hypersensitivity to perfluorcarbon or to any of the components of Luminity</td>
</tr>
<tr>
<td>Recent acute coronary syndrome or clinically unstable ischemic cardiomyopathy, including: evolving or ongoing myocardial infarction, unstable angina at rest within the last 7 days, significant worsening of cardiac symptoms within the last 7 days, recent coronary artery intervention or other factors suggesting clinical instability (for example, recent deterioration of ECG, laboratory or clinical findings), acute cardiac failure, Class III/IV cardiac failure, or severe rhythm disorders</td>
</tr>
<tr>
<td>Patients known to have right-to-left cardiac shunts, severe pulmonary hypertension (pulmonary artery pressure &gt;90 mmHg), uncontrolled systemic hypertension and in patients with GOLD Stage IV COPD, diffuse interstitial fibrosis or adult respiratory distress syndrome</td>
</tr>
<tr>
<td>Patients with cardiovascular instability where dobutamine is contraindicated</td>
</tr>
</tbody>
</table>

STUDY PROCEDURES

INTERVENTION

A flow chart of the patient work-up after presentation in our hospital is presented in Figure 7.1. Low-dose thrombolytic treatment with urokinase will be initiated following our standard institutional protocol: a catheter is placed intra-arterially in the affected artery and a bolus injection of 500,000 International Units (IU) of urokinase (Medacinase Urokinase, Medac GmbH, Hamburg, Germany) will be followed by the continuous infusion of 50,000 units of UK per hour and 9,600 IU of heparin per 24 hours. The experimental treatment consists of
(in addition to the standard thrombolytic therapy) the use of local 1.8 Mhz transdermal ultrasound (Philips iE33 Ultrasound Machine, Eindhoven, the Netherlands), and the intravenous infusion of 4 Luminity vials (total 6 mL, diluted with saline 0.9% to 40 mL total, Lantheus MI UK Limited, Newbury, Berkshire, United Kingdom) during the first hour of thrombolysis with urokinase. An ACIST VueJect (Bracco Imaging Europe B.V., the Netherlands) infusion pump will be used to infuse the 4 vials continuously. Ultrasound will be intermittently activated (3 seconds manual flash to burst microbubbles with Mechanical Index (MI) 1.08 (pulse duration 20 microseconds, frequency 1.8 Mhz, framerate 39 Hz), 7 seconds of visualization of inflow of the microbubbles at MI±0.11, at the site of occlusion during the first hour of thrombolysis. Criteria for discontinuation of the experimental treatment during the first hour will be the occurrence of any adverse events potentially related to the experimental treatment such as bleeding and allergic reactions.

**FIGURE 7.1**
FLOW CHART OF PATIENT WORK-UP AFTER PRESENTATION

<table>
<thead>
<tr>
<th>TIME SCHEME</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>At presentation</td>
<td>Information MUST trial</td>
</tr>
<tr>
<td>As soon as possible</td>
<td>ECG analysis, Informed consent</td>
</tr>
<tr>
<td>Directly after inclusion</td>
<td>Fill out SF-36, PCS questionnaire</td>
</tr>
<tr>
<td>Emergency program: First hour of treatment</td>
<td>Angiography/ placement thrombolytic catheter = IU: IV line (50k IU) + UK pump start low dose 5k IU/h</td>
</tr>
<tr>
<td>Ultrasound room: start MUST (US + bubble infusion) during 1st hour of treatment</td>
<td></td>
</tr>
<tr>
<td>After first hour of treatment</td>
<td>Continuation thrombolysis on surgical ward + measurements*</td>
</tr>
<tr>
<td>Every 6-24 hours after initiation treatment</td>
<td>Ultrasound room: Duplex Ultrasound</td>
</tr>
<tr>
<td>If signs of recanalization are observed on duplex ultrasound OR 1 time daily angiography at minimum</td>
<td>Angiography/ Angiography</td>
</tr>
<tr>
<td>Consultation vascular surgeon and interventional radiologist</td>
<td></td>
</tr>
<tr>
<td>Treatment successful, no improvement visible on angiography or clinical deterioration</td>
<td></td>
</tr>
<tr>
<td>2 weeks post-thrombolysis</td>
<td>Check site of wound + measurements</td>
</tr>
<tr>
<td>4-6 weeks, 3 months, 6 months and 1 year post-thrombolysis</td>
<td>Follow-up vascular surgeon: clinic + measurements</td>
</tr>
</tbody>
</table>

*Additional study measurements performed at the first day of thrombolysis
- Peripheral microcirculation of the skin every 15 min during first treatment hour
- ABI measurements, VAS pain assessments every 3 hours
- Duplex ultrasound every 6-24 hours during daytime
- Peripheral microcirculation measurements after 1 hours and after every duplex ultrasound

*Additional measurements performed during follow-up
- 2-4 weeks: ABI, toe pressures, VAS pain score
- 6 weeks: Walking test, ABI, prostaglandins, VAS pain, duplex ultrasound
- 3 months: ABI, toe pressures, VASpain, PCS, QoL, questionnaire
- 6 months: ABI, toe pressures, VASpain, PCS, QoL, questionnaire
- 1 year: ABI, toe pressures, VASpain, PCS, QoL, questionnaire
ASSESSMENTS

DIAGNOSTIC MEASUREMENTS

Additional diagnostic measurements during admission including ECG, duplex ultrasound, angiography and microcirculation measurements (by Laser Doppler flowmetry) will be performed as depicted in Figure 7.1.

A duplex ultrasound will be performed every 6±2 hours to monitor for signs of revascularization. When resumption of flow is visualized by duplex ultrasound, angiography will be performed to confirm flow. Angiography will be performed at least once daily as standard procedure. Outside of routine hospital working hours, angiography will only be performed in emergencies as per standard care.

A standardized pain score (Visual Analogue Scale, 1-10), and Pain Catastrophizing Scale will be recorded every 3 hours by a nurse practitioner, research fellow or surgical resident to assess pain.

FIBRINOGEN MONITORING

Following our standard institutional thrombolysis protocol, fibrinogen concentration will be checked during thrombolysis with the following criteria for treatment modification: If <1.0 g/L, the urokinase infusion rate will be lowered to 25,000 IU/h; if <0.5 g/L, thrombolysis must be aborted temporarily and replaced by normal saline infusion. Three hours following treatment modifications, fibrinogen concentration will be reevaluated and when >1.0 g/L thrombolysis will be restarted at an initial low dose urokinase of 50,000 IU/h.

POST-PROCEDURAL ANTICOAGULATION

After successful thrombolysis, the patient will be heparinized with low-molecular weight heparin (fraxiparine) dosed based on body weight: <50 kg: 2 times a day 3,800 IU (= 0.4 mL), 50-80 kg: 2 times a day 5,700 IU (= 0.6 mL), >80 kg: 2 times a day 7,600 IU (= 0.8 mL).

Concomitant therapy with coumarin derivatives will also be started at that time. Activated partial thromboplastin time (aPTT) will be measured daily during heparin treatment. The target range international normalized ratio (INR) will be 2.5 to 3.5; if this value is reached, heparinization will be stopped and coumarin treatment will be continued.

FOLLOW-UP

Outpatient follow-up will take place at specific time points for a total duration of 1 year, measurements performed during follow-up visits are depicted in Figure 7.1.

ADVERSE EVENTS

Adverse events will be recorded in detail in the electronic patient record. Any serious adverse events that occur after joining the trial will be reported to the accredited Medical Ethics Committee of our institution according to national and institutional guidelines. All adverse events will be followed up until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as in-
dicated, and/or referral to a general physician or medical specialist. An interim analysis after 10 patients will be performed and if serious adverse events have occurred, we will discuss the continuation of the study. The study will be prematurely terminated if 2 or more intracranial bleedings occur or more than 5 allergic reactions.

**STATISTICAL ANALYSIS**

Categorized epidemiologic/descriptive patient variables are summarized with frequencies and will be analyzed with Fischer’s exact test or the Pearson Chi-squared test. To avoid possible violations of the assumptions for parametric testing, such as a normal distribution pattern, we will employ non-parametric methods such as a Spearman rank correlation and a Mann-Whitney U test in the case of a skewed distribution or log transformation. For associations of two outcome measurements, we will use a correlation (Spearman rank) or single regression analysis. We will analyze the following outcomes by means of Kaplan-Meier curves: patency rate, amputation-free rate, and intervention-free rate. We will assess heterogeneity in prognostic factors as a secondary analysis by means of Chi-squared tests. All tests will be performed two-sided, and a p<0.05 will be considered to be statistically significant.

**ETHICS AND DISSEMINATION**

The study will be conducted according to the principles of the Declaration of Helsinki (Brazil, October 2013), and in accordance with the Medical Research Involving Human Subjects Act (WMO). An Investigator Site File will be produced in advance of the study conforming to institutional guidelines. Furthermore we will create Case Report Forms by using GCP and 21 CFR Part 11 compliant software, to handle patient data.

The study has been registered in the Dutch Trial Register, at the Dutch National Central Committee on Research Involving Human Subjects (CCMO) and in the European Clinical Trials Database (EudraCT) of the European Medicines Agency. Any protocol amendments during the study will be communicated and changed accordingly in the relevant registries after approval of the institutional Medical Ethics Committee. The results of this study will be submitted for publication in a peer-reviewed journal, regardless of the outcome of this study, according to the CCMO statement on publication policy. Data will also be presented at international conferences.
DISCUSSION

The Microbubbles and UltraSound-accelerated Thrombolysis (MUST) trial is a phase-II single-arm clinical trial. In this study the safety and feasibility of an experimental ultrasound technique will be investigated for the first time in patients with large peripheral arterial occlusions.

We believe that this procedure is safe and can accelerate thrombolysis, thereby allowing for reduction of thrombolytic dosage, which in turn reduces the risk of major hemorrhagic complications.

An experimental bolus therapy with microbubbles and ultrasound could accelerate thrombolysis because at high ultrasound intensities microbubbles can collapse, resulting in mechanical forces on the clot surface. The formation of small channels in the thrombus lead to exposure of a larger total surface susceptible to thrombolytics.\(^5\)

In regard to the therapeutic application of contrast agents, several studies have been performed in patients with ischemic stroke and myocardial infarction. A systematic review of sonothrombolysis shows that this treatment option improves clinical and long-term outcomes, while potentially reducing bleeding risk, in patients with ischemic stroke.\(^13\) Nevertheless, dose escalation studies show that the safety (in terms of bleeding and microemboli) needs to be further investigated before enrolling patients in phase-III trials.\(^14\) Few and heterogeneous studies examined the therapeutic application of sonothrombolysis in patients with myocardial infarction. Although pilot studies affirm safety and feasibility, the application of therapeutic ultrasound with longer pulse durations (20 microseconds vs. 5 microseconds) was reported to result in unexpected coronary vasoconstriction in a recent clinical trial.\(^15\)

Potential reported mechanisms for this effect are the summative effect of myocardial ischemia, reperfusion damage and long-pulse-duration sonoporation on endothelial damage, all leading to calcium overload.

However, patients with peripheral arterial occlusions are mostly chronic vascular patients who often have received previous treatments in the respective artery, for example thrombolytic therapy, percutaneous transluminal angioplasty, thromboembolectomy or bypass surgery. The mechanical manipulation of the vascular wall during all these treatments is extensive. Furthermore, during standard thrombolytic treatment, arteries are manipulated and perforated on purpose to insert guide wires and catheters. Hence, vascular spasms during these peripheral treatments are normal and non-threatening to the patient, in contrast to spasms in small coronary arteries.

The administration of ultrasound contrast agents has been accompanied by important discussions regarding safety concerns in the past.\(^8,16\) As a response to the occurrence of SAEs, the US Food and Drug Administration (FDA) issued a labeling change and warnings for contrast agents in 2007. Consequently, new studies on the risks of contrast agents were performed and these established their safety.\(^17\)

In regard to Luminity contrast agent dose regimens, the recommended dose for diagnostic indications is 1.3 mL dispersion added to 50 mL of sodium chloride 9 mg/mL (0.9%) or glucose 50 mg/mL (5%) solution injected over a short time-period.\(^18\) For therapeutic purposes, in large peripheral arteries there are no dose studies available. However, in our university hospital the Sonolysis study has been performed by our Cardiology department to treat acute ST elevation myocardial infarction patients with Luminity microbubbles and high mechanical ultrasound.\(^19\) The dose used was one flacon Luminity of 1.5 mL which contains
225 microliter perflutren diluted with 48.5 mL of saline 0.9% to create a 50 mL suspension. Patients were treated with 15 minutes with an infusion rate of 200 mL/h. No serious adverse events occurred. In the present study to establish a therapeutic effect in large arterial occlusion we will also infuse 1 vial per 15 minutes but we will treat patients for 60 minutes. We will use 4 flacons of 1.5 mL Luminity containing 900 microliter perflutren diluted with saline 0.9% to 40 mL total volume to be infused during 1 hour. The clinical consequences of overdose with Luminity are not known. Single doses of up to 100 microlitres dispersion/kg and multiple doses up to 150 microlitres dispersion/kg were tolerated well in Phase I clinical trials. This equals to the infusion of 12 mL (8 flacons) of Luminity dispersion. We will administer a total of 6 mL (4 flacons) of Luminity dispersion. Furthermore, we will not administer them as single bolus doses but as low-speed continuous infusion. During the experimental protocol with microbubble infusion, patients will be continuously monitored.

As with all contrast agents, the risk of anaphylactic reactions to contrast remains. Therefore, administration of contrast agents in a center with full resuscitation possibilities is mandatory. Furthermore, during the first hours of administration, monitoring of vital parameters of patients is important.

In this study, thrombolysis is performed with the fibrinolytic urokinase, which is the most used fibrinolytic agent for the treatment of peripheral arterial occlusions worldwide and is standard care in the Netherlands. Some countries use tissue plasminogen activator for this indication. A Cochrane review on the topic states that there is no evidence that (r)t-PA is more effective than urokinase for patients with peripheral arterial occlusion. If the application of microbubbles and ultrasound concomitant to catheter-directed thrombolysis is shown to be safe and technically feasible based on this phase-II trial, we anticipate a funding application for a larger randomized controlled trial with a comparative group to assess and compare efficacy of this treatment.

Although the efficacy of the currently described protocol cannot be adequately compared within this study design, we will discuss the outcomes relative to a historic control group that had previously received our standard hospital thrombolysis protocol. Successful thrombolysis is strongly predictive of amputation-free survival with vascular patency for at least one year. A longer duration of thrombolysis inevitably exposes a patient to a higher thrombolytic dose and higher risk of hemorrhage, in addition to an already increased patient burden because of prolonged bed rest. Therefore ultimately, acceleration of thrombolysis with microbubbles could benefit the patient because of a shorter therapy time, a lower risk of hemorrhagic complications and a decrease in patient burden.
ACKNOWLEDGEMENTS

We would like to thank Laura van Wieringen, Lloyd Belliot, Joyce Lu, Jorn Meekel, Ted van Schaik and Sean Matheiken for their assistance in preparation of this study.
REFERENCES


