GENERAL INTRODUCTION AND THESIS OUTLINE
Acute ischemia of the lower extremity due to peripheral arterial occlusion is a limb and life threatening disease with major impact, both for the individual patient as well as on a community level.\textsuperscript{1} In 30\% of patients with acute limb ischemia, amputation is inevitable within a year after the first presentation, while 5 year-mortality of these patients is as high as 38\%.\textsuperscript{2} Acute limb ischemia is the third leading cause of atherosclerotic cardiovascular morbidity, following coronary artery disease and stroke. The incidence of peripheral arterial occlusion has increased exponentially worldwide in the last decade, in both developed and developing countries.\textsuperscript{3} Continuous steps forward have been made in vascular disease management, including optimizing cardiovascular risk factors and medical therapy as well as developing invasive treatments. Nevertheless, an increasing life expectancy and a growing group of elderly will also result in an increasing incidence and prevalence of vascular diseases. Subsequently, an increase in burden of disease and health care costs seems inevitable. Therefore, more efficient treatment options are needed. Current treatment of acute limb ischemia of the lower extremity consists of gaining reperfusion of the occluded limb by either surgical or endovascular means, the latter includes thrombolytic therapy.

The aim of this thesis is to investigate novel protocols and techniques towards improving thrombolysis as a therapy for peripheral arterial occlusion.
1.1 THE PROBLEM: OCCLUSION OF PERIPHERAL ARTERIES LEADS TO LIMB- AND LIFE-THREATENING SITUATIONS

Oxygenated blood is transported to tissues and organs via the aorta and the peripheral arterial system. Atherosclerosis occurs when plaque accumulates inside the arterial wall. Due to progression of this disease, arteries may occlude and oxygen can no longer reach its destination (Figure 1.1). This then leads to tissue and organ ischemia which in turn may eventually lead to limb amputation or death, if not treated promptly. Therefore, fast and adequate treatment is crucial to prevent limb- and life threatening ischemia.

1.1.1 THE UNDERLYING CAUSES OF PERIPHERAL ARTERIAL OCCLUSION

Multiple cardiovascular risk factors contribute to the pathogenesis of atherosclerosis, including dyslipidemia, endothelial dysfunction, smoking, high blood pressure, and low physical activity. Derived from the Greek words ‘atherē’ (gruel, porridge) and ‘sclerosis’ (hardening) atherosclerosis implicates a slow process of hardening with simultaneous soft changes in the vascular wall. It leads to narrowing of the vascular lumen and compromises blood flow. A complex interaction of the malfunctioning local vascular endothelium and impaired blood flow can result in imbalances in hemostasis causing thrombosis (Figure 1.2). Depending on the location of thrombosis, this can lead to myocardial infarction, cerebrovascular accident or ischemia of the extremities. Patients with acute occlusion of a peripheral artery are likely to
present with several or all of the 6 P’s of arterial limb ischemia depending on the degree of ischemia: pain, pallor, polar cold, paralysis, paresthesia and pulselessness. Causes of arterial occlusion other than thrombosis on preexistent atherosclerotic arteries can be failed vascular grafts, thrombosis of an aneurysm, vascular dissection, entrapment syndrome, a hypercoagulable state, a low flow state, trauma or emboli.

**FIGURE 1.2**
PATHOPHYSIOLOGY OF PERIPHERAL ARTERIAL THROMBOSIS AS A RESULT OF ATHEROSCLEROSIS

Simplified visualization of the different stages of progression of atherosclerosis leading to an acute arterial thrombosis. Conventional- and predisposing risk factors for progression of atherosclerosis are smoking, diabetes, dyslipidemia, hypertension, advanced age, gender (male or postmenopausal women), overweight/obesity, insulin resistance, physical inactivity, family history, socioeconomic factors and ethnicity.³

Abbreviations: MMP = Matrix MetalloProteinases

Illustration used with permission from P.Broderick (modified version displayed)
1.2 | UPDATES ON TREATMENT OPTIONS FOR PERIPHERAL ARTERIAL OCCLUSIONS

Treatment of peripheral arterial occlusions consists of removal of the thrombus to restore blood flow. This can either be achieved by surgical- or pharmaco-mechanical removal, depending on the degree of ischemia: in limbs with acutely threatening ischemia, reflected by moderate sensory loss and muscle weakness at physical examination, immediate surgical revascularization is required.5

Open surgical revascularization consists of balloon catheter thromboembolectomy, endarterectomy or bypass grafting. Viable- or marginally threatened limbs, reflected by minimal sensory loss but without loss of motor function, allow for pharmaco-mechanical removal, also called catheter-directed thrombolytic therapy.

1.2.1 ADEQUATE PATIENT SELECTION AND TREATMENT INDICATION

Adequate patient selection and the right indication for treatment is vital. Unnecessary risks of bleeding could be prevented in patients that have conditions that preferably require other treatment modalities, such as patients with symptoms due to chronic ischemia that would benefit more from walking therapy, or patients with non-viable limb ischemia that should undergo amputation to prevent further deterioration and death.

Furthermore, absolute contraindications for fibrinolysis should be followed by the decision to perform an alternative treatment, and relative contraindications for fibrinolysis (see Table 1.1) should be carefully weighed. Before initiation of therapy, baseline hemostatic parameters should be evaluated in order to exclude any pre-existing coagulation disorders that could allow for easy distortion of the coagulation/fibrinolysis balance.

### TABLE 1.1 CONTRAINDICATIONS FOR CATHETER-DIRECTED THROMBOLYSIS

<table>
<thead>
<tr>
<th>ABSOLUTE CONTRAINDICATIONS</th>
<th>RELATIVE CONTRAINDICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing bleeding after failed hemostasis or active bleeding not viable to treat</td>
<td>Uncontrolled hypertension: 200 mmHg systolic or 100 mmHg diastolic blood pressure</td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td>Puncture of non-compressible vessel</td>
</tr>
<tr>
<td>Presence or development of compartment syndrome</td>
<td>Recent eye surgery</td>
</tr>
<tr>
<td>Severe limb ischemia, which in the judgment of the treating physician requires immediate operative intervention</td>
<td>Diabetic haemorrhagic retinopathy</td>
</tr>
<tr>
<td>Thrombocytopenia &lt;50 x 10^9/l</td>
<td>Neurosurgery within past 3 months</td>
</tr>
<tr>
<td>Severe thrombocytopeny</td>
<td>History of severe contrast allergy or hypersensitivity</td>
</tr>
<tr>
<td>Intracranial tumor, aneurysm or arteriovenous malformation</td>
<td>Intracranial trauma within 3 months</td>
</tr>
<tr>
<td>Trauma with visceral damage, multiple hematomas or cardiopulmonary resuscitation within past month</td>
<td>Recent (&lt;10 days) gastrointestinal bleeding</td>
</tr>
<tr>
<td>Recent (&lt;10 days) surgery or trauma</td>
<td>Established cerebrovascular event (including transient ischemic attacks within past 2 months)</td>
</tr>
<tr>
<td>Surgery of a non-compressible vascular structure &lt;2 months</td>
<td>Recent internal or non-compressible hemorrhage</td>
</tr>
<tr>
<td>Esophagus varices</td>
<td>Hepatic failure, particularly in cases with coagulopathy</td>
</tr>
<tr>
<td>Bacterial endocarditis</td>
<td>Esophagus varices</td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>Mitral valve stenosis with concomitant dilated left atrium</td>
</tr>
<tr>
<td>Mitral valve stenosis with concomitant dilated left atrium</td>
<td>Pregnancy/postpartum status</td>
</tr>
<tr>
<td>Pregnancy/postpartum status</td>
<td>Diabetic hemorrhagic retinopathy</td>
</tr>
<tr>
<td>Diabetic hemorrhagic retinopathy</td>
<td>Life expectancy &lt;1 year</td>
</tr>
<tr>
<td>Life expectancy &lt;1 year</td>
<td>Age &gt;75 years</td>
</tr>
</tbody>
</table>

Adapted from *Karnabatidis 2011* and amended based on local institutional guidelines6
1.2.2 THROMBOLYTIC THERAPY

Thrombolytic therapy was first developed in the 1970s starting with a landmark study by Dotter et al. showing successful lysis of peripheral arterial thrombosis by infusion of intra-arterial streptokinase. Due to its bacterial ancestry, repeated exposure to streptokinase can lead to significant antigenic reactions including anaphylaxis, and therefore alternative drugs were investigated. Since then urokinase (UK), derived from human urine, and (recombinant) tissue-type plasminogen activator ((r)t-PA), have increasingly become established as the first line agents for peripheral arterial thrombolysis. Intra-arterial infusion of fibrinolytic agents via a catheter has proven its efficacy in restoring blood flow by dissolving the clot (Figure 1.3). It is a less invasive alternative to surgical thromboembolectomy, and has been included in standard clinical care since the 1990s.

FIGURE 1.3
SUMMARY MODE OF ACTION FIBRINOLYTICS

Fibrinolytics, such as tissue-type Plasminogen Activator (t-PA) and urokinase-type Plasminogen Activator (u-PA), act by converting (blue arrow) the inactive proenzyme plasminogen into the active serine protease plasmin, that degrades fibrin. In red Plasminogen Activator Inhibitor (PAI) that inhibits t-PA/u-PA and alfa1-Plasmin Inhibitor (alfa1-PI) and alfa2-MacroGlobulin (alfa2-MG) that inhibit Plasmin.
1.2.3 THE ADVANTAGES AND PITFALLS OF THROMBOLYTIC THERAPY

The main advantages of thrombolytic therapy over surgical revascularization of peripheral arterial occlusions include a lower morbidity, shorter length of hospital stay, and considerably less patient discomfort. Moreover, patients with peripheral arterial occlusions frequently have concomitant comorbidities such as cerebrovascular or coronary artery disease that places them at high risk for adverse cardiovascular outcomes during and after surgery. For these patients, minimally invasive thrombolytic therapy lowers or eliminates the risks of surgical complications including perioperative risks of anesthesia and narcosis and postoperative wound complications. In addition, among this high-risk population are patients that are not eligible for surgical revascularization and for which thrombolytic therapy provides a viable treatment option. Furthermore, thrombolysis allows for more complete dissolution of outflow arteries as well as the microvasculature and for preoperative identification of underlying lesions causing thrombosis to occur. Treating patients with thrombolytic therapy as initial treatment also reduces the magnitude of potential additional surgical interventions.⁸

On the other side of the coin, a major pitfall of thrombolysis remains the risk of bleeding complications. Up to a frightening 13% of patients are faced with major bleeding complications including potentially lethal or incapacitating cerebral hemorrhages.⁹ In contrast to the lysis of small arterial occlusions in patients with myocardial infarction or cerebrovascular infarction, larger peripheral arterial occlusions require higher doses of lytic agents and infusion over a longer period of time to be dissolved. This results in a burden for the patient who has to be confined to bed for several days as well as logistic burden for high-level of care units. The treatment also requires daily evaluation by angiography with nephrotoxic contrast agents risking renal failure.

Reflecting on these matters, thrombolytic therapy is a minimally invasive alternative to surgical revascularization of peripheral arterial occlusions with several advantages over the latter, however improvements are needed towards minimizing bleeding complications and improving the efficacy to lower the treatment duration and the patient burden.
1.2.4 DEVELOPMENTS IN FIBRINOLYTIC REGIMENS AND TECHNIQUES

Since its introduction in the 1990s, several fibrinolytic agents have been used as first line agents for peripheral arterial thrombolysis. Urokinase and recombinant tissue plasminogen activator are the most effective agents but neither of these have shown to be superior in terms of efficacy and bleeding complications.\(^\text{10}\) A considerable amount of literature on a wide variety of thrombolytic agents is available, yet few randomized controlled clinical trials have been conducted and the infusion techniques and regimens are heterogeneous. A local and selective approach by intra-arterial infusion yields most efficacy and is accompanied by less bleeding complications compared to its non-selective alternative by intravenous systemic infusion of fibrinolytics.\(^\text{11}\) However, to date, the optimal dose regimen has not been established.
1.3 NEW TREATMENT OPTIONS FOR PERIPHERAL ARTERIAL OCCLUSIONS

In the past decades, several devices were developed to mechanically accelerate thrombolysis. These can roughly be divided into three distinct classes: rheolytic, rotational and mixed devices using a combined pharmacomechanical approach. Rheolytic devices utilize the Bernoulli principle and the Venturi effect to disrupt the thrombus and aspirate it via a catheter. Rotational thrombectomy functions via a rotating helix inside the catheter that produces suction and removes the thrombotic material.[12,13] A drawback of these mechanical thrombectomy techniques remains the risk of distal embolization of fragmented thrombus material resulting in limb loss. A mixed approach of pharmacological and mechanical thrombolysis is utilized to minimize the latter by using a catheter with distal and proximal balloons.[14] In the literature numerous results of these and similar devices have been reported.[12,15] Unfortunately, well-organized multicenter randomized controlled clinical trials have not been performed and data on efficacy, superiority and safety lack to provide clear indications for their application in the treatment of peripheral arterial occlusions.

1.3.1 USING ULTRASOUND TO ACCELERATE THROMBOLYSIS

Another technique uses ultrasound and its mechanical effects to achieve thrombolysis. Owing to their physical nature, ultrasonic pressure waves weaken the fibrin structure and erode the thrombus by several proposed mechanisms including acoustic cavitation, microstreaming, shear stress, intracellular microcurrents, thermal warming, and increased clot permeability.[16] A recent clinical trial investigated the efficacy and adverse events of this technique compared to routine catheter-directed thrombolysis.[17] Although the technique showed promising in accelerating thrombolysis, it still does it at the cost of a high incidence of major bleeding complications reflected in both trial arms. This emphasizes the need for a better treatment strategy by a double-edged sword: to increase the efficacy while simultaneously lower the bleeding rate.

1.3.2 CONTRAST-ENHANCED ULTRASOUND TO INCREASE THE LYtic EFFECT OF THROMBOLYTIC THERAPY

A potential accelerator of thrombolytic therapy is contrast-enhanced ultrasound. Contrast agents, consisting of 5-10µm 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.[18] 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.[19] This phenomenon is explained by the ability of microbubbles to lower the threshold energy needed for cavitation. In early stages of clinical research, contrast-enhanced ultrasound has been shown to be safe and potentially efficient as treatment for acute cerebral stroke and acute myocardial infarction.[20-22]
1.4 | THESIS OUTLINE

The first part of this thesis provides an update on thrombolytic treatment of peripheral arterial occlusions. In chapter 2 we provide an extensive overview of thrombolysis throughout the years: a systematic review of literature provides an update of all reported patient cohorts with peripheral arterial occlusions treated with catheter-directed thrombolysis since its introduction. In chapter 3 the results of a contemporary high-dose thrombolysis protocol performed on an intensive care unit are discussed with the specific aim to assess risk factors of bleeding complications and predictors of successful lysis. In chapter 4 the results of a low-dose thrombolysis protocol performed on a general ward are evaluated: it illustrates whether it equals the efficacy of a high-dose protocol and its impact on patient safety with regard to adverse events. In chapter 5, the results of a contemporary protocol in an Asian population are described, since peripheral arterial occlusions pose a globally increasing problem but are expected to increase exponentially especially in Asian populations.

In the second part of this thesis we describe novel treatments of thrombolytic therapy of peripheral arterial occlusions. We focus on the use of contrast-enhanced ultrasound, in an ultimate effort to improve thrombolytic therapy and eventually reduce the patient burden. In chapter 6 we describe an experimental pilot study using contrast-enhanced ultrasound to accelerate conventional thrombolysis in a porcine model of peripheral arterial occlusion. Chapter 7 illustrates the translation of this experimental technique from bench to bedside and will discuss the protocol of a phase-II clinical trial to investigate the safety and feasibility of this technique in patients with acute limb ischemia due to peripheral arterial occlusion. In chapter 8 we discuss an experimental setup which investigates the feasibility of combining microbubbles and an ultrasound catheter to treat peripheral arterial occlusions.

As a final novel treatment, local thrombolysis without catheter could be reality since chemical engineering allows to incorporate therapeutic agents into the microbubbles by different mechanisms. This provides the opportunity for targeted drug delivery since the compound can be unloaded at the site of interest by only local external application of ultrasound. In chapter 9 we investigate this new technique for feasibility and lytic efficacy in our porcine model of peripheral arterial occlusion.
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