SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES
10.1 SUMMARY

The first part of this thesis provided an update on thrombolytic treatment for peripheral arterial occlusions. In chapter 2 a systematic review of literature delivered an extensive overview of all reported patient cohorts with peripheral arterial occlusions treated with catheter-directed thrombolysis. Despite its minimal invasiveness, thrombolysis has several important drawbacks that require improvement, among which bleeding complications remain most detrimental.

In chapter 3 we illustrated that bleeding still is a major source of complications in a contemporary cohort treated with a high-dose urokinase protocol, which is the reason for these patients to be treated mostly within high level-of-care units. Also in other parts of the world cardiovascular disease poses an emerging problem: in metropolitan Asian life for example, extended life expectancies and high rates of smoking cause an exponentially increasing incidence of cardiovascular diseases. Since limited literature is available, chapter 5 showed the treatment results of an Asian cohort and demonstrates comparable treatment success rates to Western cohorts; however, also in this population, even higher rates of bleeding complications are hazardous and remain a detrimental drawback of this treatment.

However, chapter 4 suggests that a low-dose protocol on a standard level-of-care ward is equally effective in terms of treatment outcome but without the occurrence of major bleeding complications. This could implicate a logistic shift in thrombolytic therapy that would not only relieve the patient burden, but also the logistic and economic burden of thrombolytic treatment. Although accompanied by less bleeding complications and equal effectiveness in terms of clinical success and limb salvage rates, low-dose thrombolysis takes longer to succeed compared to its high-dose equivalent. The search for an optimal dose (highest efficacy with an acceptable rate of bleeding complications) continues, but always at a cost: a higher dose means faster lysis but more bleeding complications, a lower dose means less bleeding complications but a prolonged treatment time. Therefore, improving the treatment without increasing the fibrinolytic dose is key. This could be achieved by technical innovations in catheters and the use of additional techniques, such as introduced and described in chapter 1.

In the second part of this thesis the potential application and benefit of contrast-enhanced ultrasound as novel treatment of peripheral arterial occlusions has been investigated. In chapter 6 an experimental pilot study has been addressed using contrast-enhanced ultrasound to accelerate conventional thrombolysis in a porcine model of peripheral arterial occlusion, showing that the technique is feasible for this indication. Chapter 7 translated this experimental technique from bench to bedside in a protocol of a phase-II clinical trial currently running to investigate the safety and feasibility of this technique for peripheral arterial occlusions in humans. In chapter 8 we discussed an experimental setup in a porcine model, which investigates the feasibility of combining microbubbles and an ultrasound catheter to treat peripheral arterial occlusions. One step further into future applications of this technique is targeted drug delivery by using the microbubble as a carrier compound for the fibrinolytic. In this way, an invasive intra-arterial catheter would be redundant and treatment could be performed with an intravenous injection only. Chapter 9 illustrated the feasibility and efficacy of this technique in our experimental porcine model of peripheral arterial occlusion.
10.2 GENERAL DISCUSSION

The aim of this thesis was to investigate novel protocols and techniques towards improving thrombolysis as a therapy for peripheral arterial occlusion. In this chapter, we will discuss the contents of this thesis and the available literature to this end in analogy with the two parts of this thesis: updates on existing (10.2.1) and novel (10.2.2) treatments.

10.2.1 IMPROVING THROMBOLYSIS OF PERIPHERAL ARTERIAL OCCLUSIONS USING EXISTING TREATMENT REGIMES

Despite its minimal invasiveness, current thrombolytic treatment has several important drawbacks that require improvement, among which bleeding complications remain the most important. We will discuss the causes of bleeding complications during thrombolysis and suggestions how to minimize them in the next paragraphs.

CAUSES OF BLEEDING COMPLICATIONS

The most common causes of bleeding complications are mechanical trauma at the insertion site of the catheter and derailment of the coagulation-fibrinolysis balance. The latter can cause bleedings at distant locations of which intracranial hemorrhage is the most detrimental. In addition, different plasminogen activators and anticoagulant doses render different rates of bleeding complications.

MECHANICAL TRAUMA

In this thesis we observed bleeding at the puncture site of the leg as the most occurring bleeding complication in patients treated with catheter-directed thrombolysis. Localized bleeding complications at the site of catheter insertion in the groin are common during thrombolytic treatment and can be attributed to catheter placement and mechanical manipulation or luxation of the catheter in situ. However, remote bleedings at other sites also occurred that cannot be attributed to mechanical trauma; such as hematuria, retroperitoneal bleeding and intracranial hemorrhage. Distant bleeding complications could be caused by a derailment of the coagulation-fibrinolysis balance.

DERAILMENT OF THE COAGULATION-FIBRINOLYSIS BALANCE

The coagulation-fibrinolysis balance in the human body ensures that when small vascular defects or microvascular bleedings occur, platelets in conjunction with the coagulation cascade fixes them locally. On the other hand, when small blood clots occur, the intrinsic fibrinolytic process may dissolve them. However, if this balance is derailed systemically to either side major bleeding or thrombosis can occur, respectively. Bleeding can occur at any site but in particular at sites where the vascular wall is fragile, such as the microvasculature in the kidneys (hematuria) and the brain (intracranial bleeding). The higher risk of bleeding is allegedly caused by the increased lysis of hemostatic plugs.1

Numerous factors influence an already complex hemostatic/hemorrhagic balance but the clinically most relevant baseline parameters such as hypertension upon admission and advanced age are independently associated with a higher risk of intracranial bleeding.2
addition, African-American race, female gender and a low body weight have been reported to be independently associated with intracranial bleeding. In this thesis we demonstrated that thrombolysis of peripheral arterial occlusions in an Asian population is accompanied by a higher rate of bleeding complication than typically reported in European cohorts. This might be due to the fact that Asians have a lower body mass index, but Asian ethnicity has also been reported to be an independent predictor for major bleeding complications in patients with ST-segment elevation myocardial infarction and also Asian stroke patients receiving thrombolytic therapy faced significantly higher risk for intracranial bleeding relative to other race/ethnic groups.

The exact mechanism for this increased risk of bleeding in Asians remains elusive. Furthermore, the risk of bleeding due to mechanical trauma is higher when the coagulation-fibrinolysis balance is derailed.

In clinical practice the coagulation-fibrinolysis balance is assessed by monitoring the plasma fibrinogen levels. In search for a cut-off value, various studies have been performed over the years. Based on several studies in the 1990s, fibrinogen levels <1.0 g/L are considered to yield a significantly higher risk of bleeding complications and for patients with these levels, the advice has been to halt thrombolysis therapy. However, literature lacks prospective evidence that halting therapy above this fibrinogen threshold decreases the risk of bleeding; also, fibrinogen monitoring is not standard clinical practice. Moreover, an apparent normal fibrinogen level can underestimate bleeding risk in the course of fibrinolytic therapy due to accumulation of fragment X, one of several fibrin degradation products that is similar to a fibrinogen monomer but is more fragile.

**DIFFERENT PLASMINOGEN ACTIVATORS HAVE DIFFERENT EFFECTS**

On a molecular level, a systemic lytic state due to plasminogen activation might be expected to be more outspoken for less-fibrin-specific fibrinolytic agents, i.e. agents like streptokinase and urokinase, as compared to higher fibrin-specific plasminogen activators like alteplase, reteplase and tenecteplase. The latter activate plasminogen mostly when bound to fibrin and are inactive when fibrin is absent. Based on the high thrombus specific activity of the latter, it could be expected that these provide higher efficacy in lysis of thrombi, while providing a better safety profile. Surprisingly however, despite extensive clinical evaluation of these thrombus specific plasminogen activators, the expected better clinical efficacy and safety could not be demonstrated. On the contrary, in the treatment of patients with acute myocardial infarction, those few recombinant plasminogen-activating agents that have been studied in large-scale trials have shown a potential greater bleeding risk. This could be explained by a combination of the higher half-life of these agents and their higher fibrin affinity that could work counterproductive. It might exhibit a great affinity to the desirable fibrin plugs that seal defects in distant vessels such as the intracranial vasculature.

**FIBRINOLYTIC- AND ANTICOAGULANT DOSE PROTOCOLS: HIGHER DOSE MEANS HIGH RISK OF BLEEDING**

When higher doses of fibrinolytics are used, the risk of bleeding complications is synchronously higher, as demonstrated in this thesis. When a high dose protocol is used, up to 17% of patients suffer bleeding complications, 9% of them major (i.e. life-threatening or requiring intervention or transfusion). This implicates clinically one should tell a patient that almost 1 out of 5 patients suffer bleeding complications and 1 out of 10 patients suffer a major bleeding as a complication of this therapy.
Besides, the periprocedural anticoagulation regimen might have an important role in bleeding risk as well. Data from the TOPAS trial suggested a significant link between the co-administration of intravenous therapeutic doses of heparin and the risk of major bleeding. Furthermore, when subtherapeutic doses of heparin were administered in concomitance of a low-dose thrombolysis protocol, APTT levels were within therapeutic ranges in the majority of patients at least once within the treatment period, which could be because of the potential synergistic effects of urokinase and heparin.

**HOW TO MINIMIZE BLEEDING COMPLICATIONS**

**CLINICAL AND HEMOSTATIC MONITORING**

In the first place, monitoring is important during thrombolytic treatment. In addition to clinical monitoring for symptoms or signs of bleeding complications (such as hematoma, swelling, pain), parameters that can be monitored in order to perform an adequate risk assessment for bleeding complications are blood pressure, plasma fibrinogen, APTT and thrombocyte count. The frequency, indication and interpretation of these measurements should be carefully weighed and chosen based on the local situation depending on fibrinolytic dose and level-of-care. As described in chapter 4 of this thesis, a low-dose thrombolysis protocol can be performed on a conventional surgical ward with comparable short (angiographic and clinical success rates) and long term outcomes (amputation-free survival) to its high-dose equivalent, but without major bleeding complications. Koraen-Smith et al. also reported a cohort of patients treated with the same rt-PA protocol in two different level-of-care settings; and concluded thrombolysis may be undertaken outside of a high dependency setting without a significantly increased risk of complications. However, pre-existing cardiac disease was an independent risk factor for transfer to a higher level of care. Nevertheless, systolic blood pressures >180 mmHg, plasma fibrinogen <0.5 mg/dL, APTT >2 times control and thrombocyte count <150 x10^6/mL should set off alarm bells and should be immediately acted upon by careful evaluation and potential alteration- or cessation of therapy.

Furthermore, dosing of fibrinolytic and concomitant anticoagulation therapy has significant effect on the risk of bleeding as has been described in the previous paragraph. Therefore, it might be advisable to be less aggressive with fibrinolytic dosing in for example frail patients, refrain from systemic heparinization and only use sub therapeutic doses of heparin over the sheath as preventive measure for pericatheter clotting.

**OPTIMIZING THERAPY DURATION**

In the second place, unnecessary continuation of thrombolysis and thus unnecessary exposure to an increased bleeding risk should be avoided by adequate clinical decision making. However, different situations demand a different discussion: high-dose therapy without any improvement on follow-up angiograms and persistence of clinical symptoms needs to be cessedated, whereas low-dose therapy with little or no improvement on follow-up angiograms but improvement of clinical symptoms could allow for an extension of thrombolytic therapy.
10.2.2 OPTIMAL THROMBOLYSIS OF PERIPHERAL ARTERIAL OCCLUSIONS USING NOVEL TREATMENT MODALITIES

Novel treatment modalities could overcome important pitfalls that are inherent to the existing treatment regimens or require further improvement. Below we will describe current and new thrombolysis techniques in short and then focus on contrast-enhanced ultrasound as novel treatment modality for patients with peripheral arterial occlusions.

LIMITED IMPROVEMENTS IN CURRENT THROMBOLYTIC THERAPY: SEARCH FOR NEW TREATMENT MODALITIES

Improving thrombolytic therapy aims at minimizing major bleeding complications while at the same time improving efficacy and lowering treatment durations. As described in the general introduction of this thesis, several developments in thrombolytic therapy have been studied including all sorts of pharmacomechanical devices and new fibrinolytics. However, while promising results have been reported and individual experiences gained some degree of success in improving efficacy, none has yet shown to be superior in a randomized controlled clinical setting. In three decades, the treatment modality has basically remained the same.

The most progress has been achieved by an increased experience in interventional techniques with newer materials and better imaging equipment, as well as improvement of patient selection based on an increased clinical experience and subgroup analyses published over the years. Nevertheless, current techniques and regimens require further improvement and refinement by continuous analysis of protocols, by adding technical innovations and by piloting their effects and potential to facilitate the best of two worlds: high efficacy and low bleeding complications. In addition, simultaneous development of new and innovative concepts to develop ground-breaking techniques on the long term is desirable.

To this end, we demonstrated the feasibility of a new technique in a porcine model of peripheral arterial occlusion: the use of contrast-enhanced ultrasound to accelerate thrombolysis.

CONTRAST-ENHANCED ULTRASOUND TO ACCELERATE THROMBOLYSIS: ITS POTENTIAL

The administration of contrast agents for ultrasound is predominantly safe: they don’t derail the hemostatic/hemorrhagic balance like fibrinolytics, they don’t impact renal function like angiography contrast agents do and they are not radioactive. Their constitution of a heavy gas core and a lipid shell allows for its therapeutic effect to be exerted, with a half-life of approximately 2-6 minutes ensured by exhalation via the lungs. All lipid components are phagocytosed and distributed to the liver and spleen via the reticuloendothelial system where they are converted to free fatty acids while synthetic components if incorporated in microbubbles to prevent coalescence (such as poly ethylene glycol) are excreted in urine.

Their current therapeutic potential in sonothrombolysis consists of enhancement of the mechanical effects of external ultrasound. It could facilitate the bolus technique used in thrombolytic therapy: by formation of microchannels in the thrombus a larger thrombus surface can readily be targeted by fibrinolytics to dissolve the thrombus. This could accelerate thrombolytic therapy and allow for lower dosages to be used while increasing lytic efficacy. This promising concept was investigated in the past decade in the field of ischemic stroke and myocardial infarction. However, the feasibility and safety of the application of this technique in larger artery occlusions has not been investigated yet. Therefore, we illustrated the
setup of a phase-II clinical trial in which 20 patients with peripheral arterial occlusions will be treated with contrast-enhanced ultrasound accelerated thrombolysis, as first in the world. By using the experimental technique evaluated in our pilot study in pigs we aimed to make the translational step from pre-clinical to clinical evaluation. The experimental technique is deployed as additional intervention to the conventional thrombolytic therapy with a low-dose protocol, allowing to have direct impact on the current treatment modality.

An extension of the method of sonothrombolysis might be the use of an ultrasound catheter that could result in more effective and local thrombolysis. A disadvantage of an external ultrasound modality, either diagnostic or therapeutic, remains operator dependency. Since the ultrasound is directed manually to focus on its target, technical success depends on its operator. By using an ultrasound catheter with ultrasonic cores along its axis and placement under angiographic control, conform conventional catheter placement, selective and very precise targeting of ultrasound to the thrombus is possible. Clinical evaluation of this technique in patients with peripheral arterial occlusions showed an effective treatment and a shorter therapy duration compared to conventional thrombolysis using a high-dose protocol. However, bleeding complications in this trial remained a major drawback also in the experimental treatment group with the ultrasound catheter. Most likely this is due to the high-dose urokinase treatment that is administered. Lowering the dose remains to be investigated, expecting that a lower dose results in less bleeding complications with still good efficacy due to the ultrasound.

A treatment using an ultrasound catheter in combination with intra-arterial infusion of microbubbles through its drug delivery lumen could increase efficacy of thrombolysis without inter-operator variability of ultrasound application. This concept has recently been illustrated with in-vitro data combined with an in-vivo model of inferior vena cava thrombosis treated with a custom-designed ultrasound catheter and lab produced microbubbles. However, no data is currently available for models of peripheral arterial occlusion. Furthermore, the effects of clinically available ultrasound catheters on FDA/EMEA allowed microbubbles are as yet unknown. We observed that microbubbles can be combined with an ultrasound catheter in-vitro and could accelerate thrombolytic treatment of peripheral arterial occlusions in a porcine model. Further research on the clinical application of this combined technique could elucidate its full potential for the treatment of patients with peripheral arterial occlusions.

CONCLUSIONS

Peripheral arterial occlusions are a global emerging disease with major health impact, threatening limb and life. The current status of thrombolytic therapy to treat these is worrying due to its detrimental bleeding complications. There is no current consensus regarding optimal fibrinolytic regimens but we observed, as could be expected, that high fibrinolytic doses are accompanied by a higher incidence of major bleeding complications. Administering of low doses decreases this risk and might be equally effective in terms of treatment success and limb salvage; but in turn it extends the treatment duration. Thrombolytic treatment may be safely performed on a conventional surgical ward if local protocols and training of personnel is instituted. The therapeutic application of mechanical catalysts such as contrast-enhanced ultrasound could accelerate thrombolysis. This newly applied technique has potential to increase thrombolytic efficacy and lower the fibrinolytic dose and treatment duration.
10.3 FUTURE PERSPECTIVES

To this day, the phase II clinical trial (MUST trial) with contrast-enhanced ultrasound to accelerate conventional thrombolysis has included >75% of patients. Due to recent market unavailability of urokinase, the fibrinolytic agent used was changed to alteplase (tPA) after 50% of patient inclusions. Although this implicated a change of the original protocol, it allows for additional investigation of the feasibility and safety of the latter combined with ultrasound contrast agent. When the study will be completed and if this new treatment technique deemed to be safe and feasible, we anticipate to perform a phase III multicenter trial in which a large group of patients will be randomized between either conventional catheter-directed thrombolysis (control group) or contrast-enhanced ultrasound accelerated thrombolysis (intervention group). In this study, we will compare its efficacy compared to conventional thrombolytic treatment. For this study, we will use an ultrasound catheter to deliver the fibrinolytic and microbubbles depending on the results of the consecutive in-vitro studies investigating the combined use.

The future potential of contrast-enhanced ultrasound might be non-invasive thrombolysis with targeted microbubbles. Microbubbles can be loaded with a drug and targeted to adhere to a specific site. Once attached to this site, they can be destroyed with high-intensity ultrasound, releasing the preloaded drug at the designated location. For thrombolytic purposes, a fibrinolytic agent could be loaded into the microbubbles. If they could be destroyed at the site of peripheral arterial occlusion and release the fibrinolytic agent near the thrombus, the use of an intra-arterial catheter for thrombolysis might be avoided. Hua et al showed promising results of targeted tPA loaded microbubbles in regards to efficacy and dosage of tPA needed in a rabbit model of small arterial occlusion. This model resembles small occluded arteries in ischemic stroke.

To objectively assess the feasibility of this less invasive treatment in large occluded arteries, as is the case in acute peripheral arterial occlusion in humans, we have performed an intervention-controlled study using a porcine model. We investigated the intravenous injection of targeted microbubbles loaded with urokinase in our porcine model of peripheral arterial thrombosis. The microbubbles were targeted to adhere to the thrombus’ surface by adding RGDS (Arg-Gly-Asp-Ser) peptide to the microbubble shell. In addition, high-intensity ultrasound was applied at the site of the thrombus in order to yield maximum local thrombolytic effect. The technique showed to be feasible and illustrates an opportunity for the future therapeutic potential of microbubbles to accelerate thrombolysis. However, in order to make the translation of this technique into clinical investigation more preclinical in-vitro experiments and knowledge about the stability, dose response and temporal efficacy are needed. If this technique would be developed and translated into clinical practice, it could implicate the redundancy of intra-arterial catheters and result in improved safety, patient comfort as well as a decrease in logistic burden and improved cost-effectiveness.
REFERENCES


