

DAMIR VAKHITOV

# Factors Affecting the Thrombolytic- Treatment-Related Outcomes in Patients with Acute Lower Limb Ischaemia



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in Patients with Acute Lower Limb Ischaemia

ACADEMIC DISSERTATION

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Tampere University, Faculty of Medicine and Health Technology  
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# Abstract

Acute lower limb ischaemia (ALLI) is a condition that often requires urgent or emergent surgical or endovascular treatment. This disorder is associated with major amputations and mortality. The aetiopathogenesis of ALLI has changed within the last decades. Currently, it mainly presents with atherosclerosis-associated thrombotic occlusions. An increasing volume of data has been published in favour of a possible bacterial inflammation that can contribute to the pathogenesis of thrombotic events, particularly in patients with atherosclerotic changes. The patients who present with ALLI are often elderly individuals with multiple comorbidities. Therefore, mini-invasive treatment modalities are preferred. Nonetheless, catheter-directed thrombolysis occasionally fails and leads to amputations. Even after successful fibrinolysis, some patients develop recurrent ischaemia and require new interventions.

The aim of this work was to study the possible aetiological issues related to ALLI from the viewpoint of bacterial deoxyribonucleic acid (DNA) presence in the thrombi. Further aims were to evaluate possible reasons behind thrombolytic treatment failure and recurrent ALLI, and to assess the long-term outcome of thrombolytic treatment.

The thrombus aspirates were obtained aseptically and examined for the presence of bacterial DNA with a quantitative polymerase chain reaction from September 2014 to October 2016. A retrospective analysis of the ALLI patients treated at Tampere and Turku University Hospitals from January 2002 to December 2015 was performed to address the other aforementioned questions.

A total of 303 cases of ALLI (159 men [52.5%]) were registered in the studies. The mean age of the patients was 71 years. A total of 58% of the native arterial and 75% of the bypass graft thrombi were identified as positive for bacterial DNA. Synthetic graft thrombi demonstrated positivity for bacterial DNA in 77.8% of the cases. Of the positive samples, 90% contained the *Streptococcus mitis* group DNA.

In patients managed with thrombolysis, an early treatment failure occurred in 23%. A delay in treatment initiation increased the risk of failure by 5% per day. Hyperlipidaemia and previous bypass grafting were also independently associated with failure. This resulted in an almost 40% risk of major amputations within the first month. Nearly 43%

of the patients developed recurrent ischaemia within a median of 40 months. Bypass graft reocclusions were predominant (65%). The absence of appropriate antiplatelet or anticoagulant treatment in native arteries and worsened tibial runoff in bypass grafts were independently associated with the risk of recurrent ALLI.

At one year, almost 80% of the patients were alive. The primary patency rate at this point for native arteries was 87%. The primary patency rates for bypass grafts ranged from 31% to 62%, with the lowest rates found in autologous vein grafts. The amputation-free survival rate was 66%.

The long-term outcomes were unfavourable. At five and ten years, 56% and approximately 30% of the patients, respectively, were alive. The survival was independently associated with the presence of atrial fibrillation and an age of over 83 years. The 10-year primary patency rates for native arteries and conduits were 18.7% and 15.2%, respectively. The amputation-free survival was independently affected by an age of over 75 years and represented a rate of 24% at 10 years.

The information on the presence of bacterial DNA in the thrombi must be interpreted with caution. Additional studies are needed to establish whether these findings are involved in the actual thrombotic process. The short-term post-thrombolytic outcomes are superior to the long-term outcomes, which are poor. Recurrent ischaemia is frequent and affects the results. Both modifiable and non-modifiable factors have an impact on the treatment outcome. They should be taken into consideration in the clinical work and further investigations.

# Tiivistelmä

Akuutti alaraajaiskemia (AAI) on tilanne, joka vaati kiireellistä tai välitöntä kirurgista tai suonensisäistä hoitoa, ja on yhteydessä lisääntyneeseen amputaatio- ja kuoleman riskiin. AAI:n etiopatogeneesi on muuttunut viime vuosikymmenien aikana. Nykyään suurin osa iskemiasta johtuu ateroskleroosin aiheuttamista valtimoahtaumista. Viimeaikaiset tutkimukset ovat osoittaneet, että bakteeritulehdus saattaa vaikuttaa tromboosiin, varsinkin potilailla, joilla on ateroskleroottisia muutoksia. AAI-potilaat ovat usein iäkkäitä ja monisairaita, jolloin liuotushoitoa pidetään hyvänä vaihtoehtona avokirurgialle. Siitä huolimatta katetriohjattu trombolyyysi saattaa epäonnistua, ja tämä voi liittyä lisääntyneisiin amputaatioihin. Onnistuneenkin liuotushoidon jälkeen joillekin potilaille kehittyä uusintaiskemia, johon liittyy uusintatoimenpiteitä. Hoidon pitkäaikaistulokset AAI-potilailla jäävät usein epäselviksi.

Väitöstutkimuksen aiheena oli selvittää AAI:n taustatekijöitä mukaan lukien uusia etiologia tekijöitä, hoidon pitkäaikaistuloksia ja niihin vaikuttavia tekijöitä.

Tutkimuksen retrospektiivisissä töissä aineistona käytettiin Tampereen ja Turun yliopistollisissa sairaaloissa hoidettujen potilaiden tietoja. Prospektiivisessä osiossa kerättiin AAI-potilaiden trombiaspiraatteja bakteeri-DNA:n tunnistamiseksi.

Yhteensä 303 AAI-tapausta (159 miestä [52,5 %]) rekisteröitiin tutkimuksiin. Potilaiden keski-ikä oli 71 vuotta. Lähes 60 % natiivivaltimoista ja 75 % ohitteista olivat bakteeri-DNA-positiivisia. Synteettiset ohitteet olivat positiivisia lähes 80 %:lla potilaista. Kaikista positiivisista valtimonäytteistä 90 % sisälsi *Streptococcus mitis* ryhmän DNA:ta. Trombolyyysillä hoidetuilla potilailla varhainen hoidon epäonnistuminen oli todettavissa 23 %:ssa tapauksista. Hyperkolesterolemia, aikaisempi ohituskirurgia ja hoidon aloituksen viivästyminen liittyivät itsenäisinä tekijöinä epäonnistumiseen, joka oli yhteydessä melkein 40 %:n amputaatoriskiin ensimmäisen kuukauden kuluessa. Uusintaiskemia ilmaantui melkein 43 %:lle potilaista 40 kk:n medianiseurannan aikana. Ohitteiden uusintatukoksia esiintyi merkitsevästi useammin natiivivaltimotapahtumiin verrattuna. Asianmukaisen verenohennus- tai antiaggregatorisen lääkityksen puute sekä säärivaltimoiden ulosvirtauksen heikkous ennustivat itsenäisesti uusintaiskemian kehittymistä.

Yhden vuoden kohdalla lähes 80 % potilaista oli elossa. Primaarinen aukipysyvyys natiivivaltimoissa oli 87 %. Ohitteiden aukipysyvyys tällä ajankohdalla oli 31 %–62 %. Amputaatiovapaa elossaolo oli 66 %. Pitkäaikaistulokset olivat epäsuotuisia. Kymmenen vuoden kohdalla noin 30 % potilaista oli elossa. Eteisvärinä ja korkea ikä (yli 83 vuotta) ennustivat itsenäisesti huonoa eloonjäämistä. Primaarinen aukipysyvyys oli natiivivaltimoissa 18,7 % ja ohitteissa 15,2 %. Yli 75 vuoden ikä vaikutti itsenäisesti amputaatiovapaaseen elossaoloon ollen 24 % kymmenen vuoden kohdalla.

Huolimatta siitä, että bakteereiden DNA:ta löydettiin trombiaspiraateista, tietoon täytyisi suhtautua varovaisesti, sillä syy-yhteyksien osoittaminen vaatii lisätutkimuksia. Katetriohjatun trombolyyysin tulokset lyhyellä aikavälillä ovat pitkäaikaistuloksia paremmat. Uusintatapahtumat ovat yleisiä, ja pitkäaikaisennuste on epäsuotuisa. Vain osaan hoidon tuloksiin vaikuttavista tekijöistä voidaan vaikuttaa. Nämä tekijät tulisi huomioida sekä kliinisessä työssä että tulevilla tutkimuksilla.

## Original Publications

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# Abbreviations

ABI	Ankle-brachial pressure index
AF	Atrial fibrillation
ALLI	Acute lower limb ischaemia
ASA	Acetylsalicylic acid
CDT	Catheter-directed thrombolysis
CS	Compartment syndrome
CTA	Computed tomography angiography
DNA	Deoxyribonucleic acid
DSA	Digital subtraction angiography
HR	Hazard ratio
MRI	Magnetic resonance imaging
OR	Odds ratio
PAD	Peripheral arterial disease
qPCR	Quantitative polymerase chain reaction
RALLI	Recurrent acute lower limb ischaemia
RHD	Rheumatic heart disease
<i>S. mitis</i>	<i>Streptococcus mitis</i>
tPA	Tissue plasminogen activator

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# 1 Introduction

Acute lower limb ischaemia is a condition caused by a sudden restriction of blood circulation in tissues lasting 14 days or less (Gilliland et al. 2017). It results in a lack of oxygen and nutrient supply to the affected limb and may potentially threaten the viability of the lower extremity.

Despite the relatively high technical success rate of the thrombolytic treatment that exceeds 70% (Acar et al. 2013), the early amputation rates remain high. They range from 10% to 30% in the short term (Byrne et al. 2014; Koraen et al. 2011; Norgren et al. 2007). The reasons for this could be partially explained by treatment failure, along with underlying comorbid conditions. The threat, however, is not only limited to the viability of the limb. These patients are at a high risk of mortality (Van Damme et al. 2018).

The effective treatment of rheumatic heart disease (RHD) and endocarditis in particular has reduced the incidence of embolic events (O'Connell & Quiñones-Baldrich 2009). With the ageing population (Van Damme et al. 2018), the prevalence of peripheral arterial disease (PAD) has, however, increased (Criqui & Aboyans 2015). Diabetes, chronic renal insufficiency, smoking and hypertension are significantly associated with PAD and increase the likelihood of its presence (Eraso et al. 2014). Therefore, more complex conditions may turn ALLI into a multidisciplinary situation. The circulation restriction in such circumstances leads to systemic electrolyte abnormalities that impair the renal and cardiopulmonary functions. Furthermore, a sudden successful reperfusion may, in some cases, worsen the patient's condition, owing to the release of toxic substances into the circulation. Whether the patient is in the end-of-life stage and whether an invasive approach should still be undertaken can often pose a clinical challenge.

Recent investigations have suggested the possible role of bacterial inflammation in the aetiology and pathogenesis of cardiovascular diseases (Pessi et al. 2013; Pyysalo et al. 2016; Renko et al. 2013). The presence and role of such microorganisms in the ALLI setting have not been studied. The issue can appear relevant, since certain bacterial species are known

for their ability to bind platelets (Bensing et al. 2001) and, possibly, contribute to thrombus formation.

Despite innovations in science and engineering, as well as in diagnostic modalities and treatment, the morbidity and mortality after ALLI remain high (Van Damme et al. 2018). During the last decades, the aetiopathological characteristics of ALLI have been changing (Van Damme & Limet 2005), which makes it interesting to assess the problem from the viewpoints of possible bacterial involvement and factors that impact on treatment failure and affect the outcomes in the long term.

## 2 Review of the Literature

### 2.1 Epidemiology and aetiological aspects of acute lower limb ischaemia (ALLI)

The information on the incidence of acute lower limb ischaemia is insufficient. According to Dormandy and colleagues (1999), it is estimated to constitute 10%–16% of the vascular workload, with a reported incidence of 14 per 100,000 in the general population. The number of hospital admissions because of ALLI has decreased from 45.7 per 100,000 in the 1990s to 26 per 100,000 in the 2000s, as reported by Baril and colleagues (2014). ALLI is equally distributed between women and men. This pathological condition mainly affects the older population with a median age of 75 years (Smith & Lilie 2019). The risk factors for ALLI include increasing age, smoking, diabetes mellitus and hypertension (Smith & Lilie 2019).

Earlier, when RHD could not be properly managed, the condition was often complicated with the development of peripheral arterial thromboembolism. Alexander Fleming discovered penicillin in 1928 (Fleming 1929), but it took another 17 years for its mass production and distribution to be launched (Aminov 2010). Jay McLean at Johns Hopkins discovered unfractionated heparin in 1918 (introduced in clinical practice in 1940s) (Franchini et al. 2016), and Karl Link synthesized warfarin in 1948 (in clinical practice in 1950s) (Pirmohamed 2006). In developed countries, these innovations have reduced the incidence and prevalence of RHD and subsequent embolism. Nevertheless, these conditions are still recognized as major causes of morbidity and mortality in developing nations, accounting for approximately 233,000 deaths annually (Carapetis et al. 2005). With an ageing population, the presentation of ALLI due to thrombosis becomes more common and, in some series from the developed countries, exceeds 60% of all cases (Grip et al. 2014).

## 2.2 Pathogenesis of ALLI

The main reasons of ALLI are thrombosis and embolism. Acute lower limb ischaemia may also occur as a result of trauma, dissections, iatrogenic injuries, entrapment or cysts, as well as a hypercoagulable state, aneurysm thrombosis and some other rare conditions related to a sudden restriction in limb perfusion (Acar et al. 2013). It is noteworthy that the differential diagnosis of these causes may be difficult. Even after a thorough clinical examination, the distinction between the two entities can be challenging, “since ischaemia can be the consequence of arterial embolism in a diseased atherosclerotic artery” (Van Damme & Limet 2005).

### 2.2.1 Embolism

The word “embolus” comes from Greek and means “a plug”. An embolus usually refers to a blood clot or sometimes an air bubble, pieces of adipose or atherosclerotic tissues that are spread through the blood stream and result in blood vessel occlusion or stenosis. Arterial thromboembolism predominantly occurs owing to cardiac reasons, with atrial fibrillation as the most frequent one (Lyaker et al. 2013), causing approximately 67% of all embolic events (Van Damme & Limet 2005). Myocardial infarction is less common (12%), while rheumatic valvulopathy (3%) and prosthetic valve-related emboli (2%) are the least probable cardiac reasons of embolism (Van Damme & Limet 2005). According to the same author, other embolic sources would include artery-originated emboli (5%), paradoxical emboli from the venous system that pass through a patent foramen ovale into the arterial system (1%), and emboli of an unknown origin (10%). Emboli usually travel through the large-diameter vessels unimpeded and naturally occlude smaller peripheral arteries. Although large-sized emboli may lodge in the aortic bifurcation, the most commonly affected part of the body is the lower limb (Abbott et al. 1982), with the occlusions found in the femoral bifurcation in over a half of the cases (Table 1) (Van Damme & Limet 2005).

Table 1. Anatomical location of lower limb emboli. Translated and modified from French. Used with the permission granted by Dr. H. Van Damme. Service de Chirurgie Cardiovasculaire et Thoracique, CHU Sart Tilman, 4000 Liège, Belgique (Van Damme & Limet 2005)

Anatomical location	Incidence (%)
Aorto-iliac segment	8%
Iliac bifurcation	12%
Femoral bifurcation	55%
Popliteal artery	20%
Tibial artery	5%

Patients presenting with embolic events usually have healthy arteries and insufficient collateral circulation (Figure 1). Once the blood supply to the extremity through an occluded vessel is interrupted, the patient presents with an acute pain and sensorimotor deficit. The clinical situation may worsen due to the development of secondary thrombotic occlusions both proximally and distally to the embolic obstruction.

Due to the achievements in medicine and to more efficient treatment of RHD, endocarditis and congenital heart conditions (O’Connell & Quiñones-Baldrich 2009), the incidence of embolism has decreased over time (Figure 2) (Van Damme & Limet 2005).

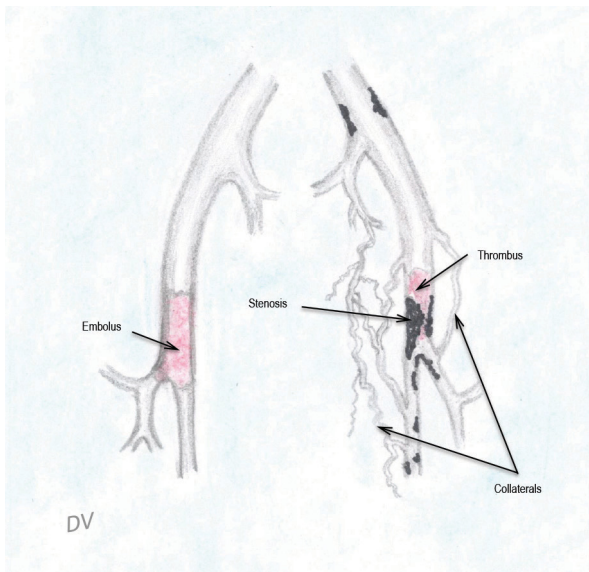


Figure 1. A schematic illustration of embolism (left) and thrombosis in situ (right). ©Damir Vakhitov

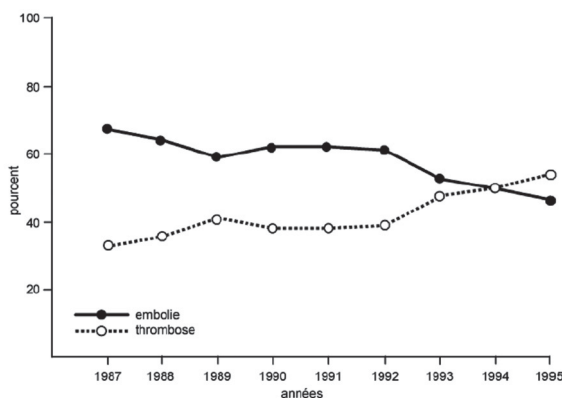


Figure 2. The evolution of the causes of acute lower limb arterial obstructions over time. The x axis demonstrates years and the y axis percentages. The black curve represents embolism and the dashed curve thrombosis. Used with the permission granted by Dr. H. Van Damme. Service de Chirurgie Cardiovasculaire et Thoracique, CHU Sart Tilman, 4000 Liège, Belgique (Van Damme & Limet 2005)

## 2.2.2 Thrombosis

Conversely, the incidence of arterial and bypass graft thrombosis has increased over time (Van Damme & Limet 2005) and currently outnumbers that of embolic events (Grip et al. 2017). The progression of underlying atherosclerosis and subsequent development of an arterial stenosis or occlusion may predispose PAD patients to thrombotic events. Furthermore, aneurysms, hypotension, hypercoagulable states and hypovolaemia can also contribute to the pathogenesis of thrombosis. Additionally, trauma, arterial dissections and malignancy may also play a potential role in the development of thrombotic occlusions (O'Connell & Quiñones-Baldrich 2009).

Various reasons lead to platelet aggregation and subsequent thrombosis *in situ*. A critical atherosclerotic narrowing of the arterial lumen is one of them. These patients often have a history of chronic ischaemia (*e.g.* claudication). However, an acute onset of the symptoms is also possible. Generally, within several hours from the onset, some patients may experience symptom relief due to the “opening” of collaterals. Another possible reason is an atherosclerotic plaque rupture, also believed to induce thrombosis (Earnshaw 2001). The mechanisms are not completely understood. In the coronary vessels, however, a chronic low-grade bacterial inflammation could contribute to these processes (Pessi et al. 2013). Interestingly, such bacteria are found in the oral cavity and could be haematogenically spread to the plaque especially after dental procedures (Pessi et al. 2013).

Hypercoagulable states may develop as a result of congenital or acquired disorders (Khan & Dickerman 2006). Among these are thrombophilias of different types, such as sticky platelet syndrome, antiphospholipid antibody syndrome, cancer-related hypercoagulability and a heparin-induced thrombocytopenia.

Arterial dissections often occur owing to trauma or systemic connective tissue disorders. As a result of mechanical obstruction, the blood supply to the limb is diminished, leading to thrombosis development.

## 2.2.3 Occlusions of bypass grafts

With the development of vascular services in developed countries, the incidence of bypass graft occlusions has increased and, in some populations, reached 15% (Campbell et al. 1998). Bypass graft patients usually present with thrombosis rather than embolism. The management of these conditions is similar to that of native arterial acute limb ischaemia.

## 2.2.4 Contribution of bacteria to the thrombotic process

The initiation and progression of atherosclerotic lesions involves a chronic inflammatory response driven by oxidized low-density lipoprotein. It is characterized by an inability to



resolve the inflammation, leading to advanced atherosclerotic lesions (Virmani et al. 2005). The possibility that bacterial infections might directly or indirectly contribute to this process is still under investigation (Rosenfeld & Campbell 2011). The variety of bacterial species possibly involved in atherosclerotic plaque progression is wide. It comprises bacteria of the gastrointestinal tract, pulmonary micro-organisms, as well as pathogens of dental and periodontal origin (Olsen 2008; Rosenfeld & Campbell 2011). Mainly deoxyribonucleic acid (DNA) of the *Streptococcus mitis* group (*S. mitis* group) was revealed in thrombus aspirates from myocardial infarction patients (Pessi et al. 2013) or patients with cerebral aneurysms (Pyysalo et al. 2016; Pyysalo et al. 2013). These microorganisms inhabit the oral cavity but can also participate in the formation of dental biofilms that calcify into dental plaques. The streptococci are common agents in infective endocarditis and septicaemia (Kerrigan & Cox 2012). They possess the ability to bind platelets (Bensing et al. 2001) and adhere to an endothelial surface (Bensing et al. 2001; Leishman et al. 2010; Mitchell et al. 2007), thus possibly participating in thrombosis.

### 2.3 Classification of ALLI

ALLI can be classified in accordance with the aetiological factors – thrombosis or embolism. Unfortunately, whether an occlusion is an embolus or thrombus cannot always be proven definitively (Van Damme & Limet 2005). Besides, it has become clear that a classification based on the severity of ischaemic changes would be more valuable to determine the urgency of required treatment.

The Society for Vascular Surgery and the International Society for Cardiovascular Surgery have established definitions based on the severity of ischaemia (Rutherford et al. 1997). These standards were later altered by the Trans-Atlantic Inter-Society Consensus, according to which ALLI can be defined as a sudden decrease in limb perfusion, causing a potential threat to the viability of a limb (Norgren et al. 2007).

ALLI may be asymptomatic in the presence of well-developed collaterals. In their absence or insufficient presence, sensory loss, followed by a motor deficit, will develop and lead to muscle weakness and, finally, skin changes (Figures 3–5, Table 2).



Figure 3. Category IIa ALLI (patient's right leg). (Table 2).  
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Figure 4. Category IIb ALLI (patient's right leg). (Table 2)  
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Figure 5. Category III ALLI. (Table 2)  
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Table 2. Classification of ALLI. (Rutherford et al. 1997)

Category	Description/prognosis	Findings		Doppler Signals	
		Sensory loss	Muscle weakness	Arterial	Venous
I. Viable	Not immediately threatened	None	None	Audible	Audible
II. Threatened					
a. Marginally	Salvageable if promptly treated	Minimal (toes) or none	None	Inaudible	Audible
b. Immediately	Salvageable with immediate revascularization	More than toes, rest pain	Mild, moderate	Inaudible	Audible
III. Irreversible	Major tissue loss or permanent nerve damage inevitable	Profound, anaesthetic	Profound, paralysis	Inaudible	Inaudible

## 2.4 Diagnostic imaging

A clinical examination often provides a physician with the required information. Therefore, radiological examinations may not be routinely needed. Several options of diagnostic imaging are, nonetheless, available, if a prompt diagnosis cannot be made. The role of minimally or non-invasive methods is evolving. Digital subtraction angiography (DSA), however, remains the gold standard (Weiss et al. 2017). It is the only diagnostic modality that permits the simultaneous treatment of acute occlusions (Weiss et al. 2017).

Nevertheless, conventional contrast angiography seems to be inferior in its accuracy in comparison with magnetic resonance angiography (MRI) (Lapeyre et al. 2005).

The diagnostic performance of MRI in PAD patients is high, with a sensitivity of 94.7% and a specificity of 95.6% (Menke 2010). In addition, the accuracy of MRI has been shown to be superior to that of computed tomography for examining below-the-knee vessels (Young et al. 2013). In a recent report (Varga-Szemes et al. 2017), non-contrast quiescent-interval single-shot magnetic resonance angiography provided high diagnostic accuracy in comparison with DSA in PAD patients. The authors suggested that such an imaging modality obviated the need for contrast administration. With the need for urgent diagnosis and intervention, the role of MRI in ALLI may, however, be limited (Fernando & Bakhos 2017).

The diagnostic performance of computed tomography angiography (CTA) is also high. The sensitivity and specificity of this method for detecting stenosis of over 50% are largely comparable to those of contrast-enhanced MRI. Nonetheless, a separate imaging technique for detecting lesions of a tibial segment by MRI is preferred (Jens et al. 2013). CTA is, however, widely used when MRI is unavailable, undesirable due to claustrophobia, or contraindicated (pacemaker, insulin pump, metallic foreign bodies, gastric reflux device and some others).

Duplex ultrasonography is a non-invasive method, the diagnostic precision of which is, however, inferior to that of MRI, contrast angiography or CTA (Collins et al. 2007). Nevertheless, in expert hands, the method provides acceptable accuracy, particularly for the diagnostics of the above-the-knee arteries.

## 2.5 Treatment of ALLI

### 2.5.1 Conservative treatment of ALLI

Anticoagulants have been administered to patients with ALLI for at least several decades. Blaisdell F. and colleagues (1978) suggested that high-dose heparin therapy could be helpful in conjunction with delayed interventions on ALLI patients. Anticoagulation helps to reduce or stop the propagation of thrombosis, to prevent new embolic events and to maintain collateral circulation. In their 2016 guidelines, the American Heart Association and the American College of Cardiology suggested systemic anticoagulation with heparin unless contraindicated (Gerhard-Herman et al. 2017).

Berridge and colleagues studied the benefits of oxygenation in 1989. They found that “continuous oxygen inhalation during acute ischaemia may improve tissue nutrition before, during and after definitive treatment” (Berridge et al. 1989). Apart from that, there is currently no evidence that vasoactive drugs would be of clinical benefit to patients with ALLI.

It is obvious that the management of ALLI with anticoagulation alone may not be a durable option in most cases. This treatment modality is occasionally reserved for those with multiple severe comorbidities and of advanced age, when other definitive surgical or endovascular procedures cannot be offered and the patient's life expectancy is otherwise short.

## 2.5.2 Modern surgical treatment

After 1961, when Thomas Fogarty introduced a balloon catheter for blood clot removal, surgical revascularization became the main option in acute limb ischaemia treatment (Figure 6). The procedure is relatively quick, and it can be performed under local anaesthesia. Despite the decrease in the incidence of embolic events over time, the limb salvage and post-operative survival rates have not improved (Ljungman et al. 1996). The life expectancy in developed countries during the last decades has increased, and the profile of ALLI has changed. The patients increasingly present with thrombosis (Van Damme & Limet 2005; Ljungman et al. 1996), which makes the management of such acute ischaemic conditions more complex. Additional open, endovascular or hybrid procedures that include both open and endovascular modalities are often needed to achieve adequate distal perfusion (Figure 7) (Byrne et al. 2014). The management of ALLI in hybrid operation suites could be recommended for that reason.

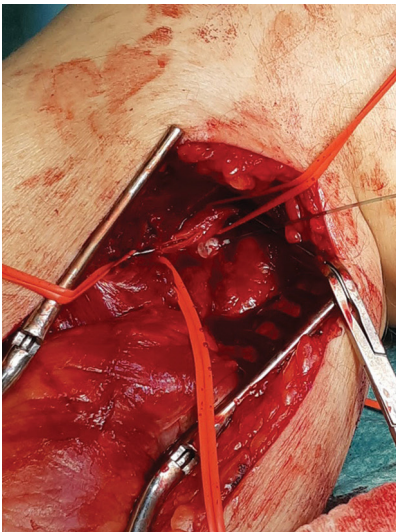


Figure 6. Small-sized emboli are removed from the tibial arteries with a Fogarty balloon catheter using the distal popliteal artery access. ©Damir Vakhitov

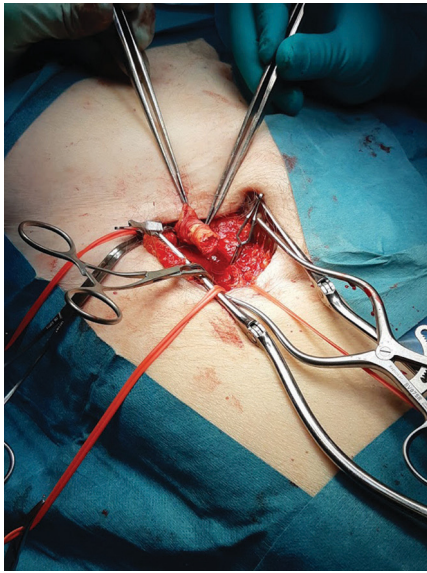


Figure 7. A severe stenosis of the common femoral artery treated by means of eversion endarterectomy. ©Damir Vakhitov

### 2.5.3 Endovascular treatment

In 1933, W. Tillett and R. Garner at Johns Hopkins Medical School in Baltimore, MD, found certain strains of *Streptococcus haemolyticus* to be capable of the rapid dissolution of a normal human fibrin clot (Tillett 1933). The first clinical reports on the use of thrombolysis were also published in the USA and date back to the 1950s. At that time, the reports concerned acute coronary syndrome treatment with massive intravenous streptokinase therapy (Fletcher et al. 1958). The risk of haemorrhagic complications was high, since large amounts of thrombolytics were required. A significant step forward was taken with the introduction of intracoronary fibrinolytics administration. The pioneers of this innovation were E. Chazov in the Soviet Union and K. Rentrop in West Germany (Chazov et al. 1976; Rentrop et al. 1979). Years of experimental work resulted in improved treatment regimens, better instrumentation and lower peri-procedural risks. This allowed the method to be applied not only in the field of cardiology but also in the management of acute peripheral arterial occlusions of the upper and lower extremities.

The modern endoluminal treatment of ALLI is based on the use of pharmacological intra-arterial thrombolysis with several technical variations. The method in its current form has been available for approximately 30 years. This modality offers relatively high technical success rates that exceed 70% (Byrne et al. 2014). In order to restore sufficient circulation and tissue perfusion, additional endovascular (balloon dilatation and stenting) or open procedures may be needed upon the completion of fibrinolytic therapy or, occasionally, within the treatment course.

Mechanical aspiration thrombectomy could be considered alternatively. The potential advantage of this method is that the procedure can be followed by thrombolysis, if the



aspiration attempts fail. The available data on the use of aspiration thrombectomy in ALLI is, however, insufficient, and no definitive conclusions can be drawn at this point. According to the recent study from Australia (Kwok et al. 2018), which included 15 patients treated with aspiration thrombectomy and 27 patients treated with catheter-directed thrombolysis (CDT), the primary technical success rates were 53% versus 89%, respectively. The authors concluded that aspiration thrombectomy could reduce the need for CDT (Kwok et al. 2018).

## 2.5.4 Amputation as a primary treatment option in ALLI

In general, major amputations are performed on patients with Class III ALLI when further revascularization is not an option due to irreversible and permanent ischaemic changes. Furthermore, amputations could be considered after failed revascularization attempts in patients with otherwise non-salvageable limbs or those in whom revascularization cannot be performed due to anatomic factors (Abou-Zamzam et al. 2003).

## 2.5.5 Open surgery versus thrombolysis

A comparison of CDT and conventional surgical modalities for the treatment of ALLI may appear questionable due to the diverse clinical presentations and a wide repertoire of surgical procedures used. Nevertheless, a number of randomized trials have succeeded in performing such an evaluation (The Stile Investigators 1994; Ouriel et al. 1994; Ouriel et al. 1996; Ouriel et al. 1998). The increased risk of ongoing ischaemia, bleeding and stroke was associated with CDT (Berridge et al. 2002). The studies, however, demonstrated no difference in limb salvage or death rates at thirty days, six months or one year (Berridge et al. 2002). A recent study from Sweden (Grip et al. 2018), which included over 6,700 patients after propensity score matching, concluded that, although the primary endovascular approach was equally effective as open surgery in regard to limb salvage, it was associated with reduced mortality rates at thirty days and after one year post-procedurally.

## 2.6 Catheter-directed thrombolysis (CDT)

### 2.6.1 Thrombolytics

There are currently several types of thrombolytic agents that can be classified according to their generations (Ali et al. 2014) (Table 3).

Table 3. Generations of thrombolytic agents. Table modified and simplified from Ali et al. (2014)

Generation of thrombolytic drugs	Thrombolytic agent
First generation	Streptokinase urokinase
Second generation	recombinant tissue plasminogen activator (tPA) - Alteplase Tenecteplase
Third generation	Reteplase Monteplase Lanoteplase Pamiteplase

Streptokinase was the first thrombolytic agent used in humans. The tissue plasminogen activator (tPA), however, seems to be safer and to offer lower systemic effects when administered as a low-dose intra-arterial infusion (Berridge et al. 1989). There is no strong evidence that tPA is more effective than urokinase. There is, however, evidence that the thrombolytic treatment might be more rapid with tPA (Robertson et al. 2013); see Table 4. Reteplase, a representative of third-generation thrombolytic drugs, is similar to tPA but has a longer half-life and superior penetration ability. Tenecteplase is also similar to Alteplase but with better binding affinity to fibrin and an even longer half-life than that of Reteplase (Karnabatidis et al. 2011); see Table 4. The reports on the use of these newer agents in ALLI are, however, few. According to the available data, the results of treatment with reteplase are acceptable and promising (Davidian et al. 2000), but a controlled comparison to other thrombolytic drugs is, however, needed before a definitive conclusion could be drawn (Ouriel et al. 2000).

Table 4. The elimination half-life of thrombolytics. Table modified from Ali et al. (2014)

	stroitokinase	urokinase	Alteplase	Reteplase	Tenecteplase
Plasma half-life (min)	18	15	4–8	11–14	20

## 2.6.2 Physiology of fibrinolysis

A schematic model of fibrinolysis is demonstrated in Figure 8. Urokinase and tPA convert plasminogen into the active form – plasmin. Plasmin turns fibrin into degradation products that contain polypeptides and amino acids. Both tPA and urokinase could be inhibited by plasminogen activator inhibitor-1 and -2. On the other hand, alpha 2-antiplasmin and alpha 2-macroglobulin can inactivate plasmin. At the same time, thrombin-activatable fibrinolysis inhibitor may reduce the activity of plasmin.



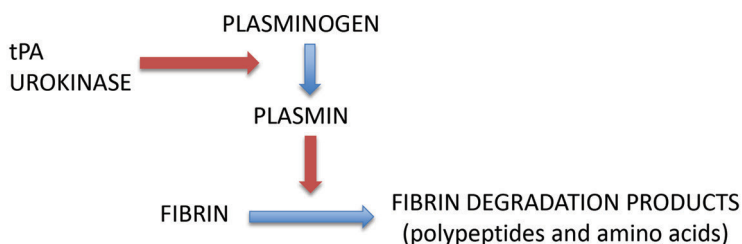


Figure 8. A simplified schematic model of fibrinolysis. ©Damir Vakhitov

### 2.6.3 Contraindications for CDT

The potential candidates for CDT are evaluated for specific contraindications, which can be divided into absolute and relative. Although slight variations between the available protocols might occur, the general contraindications are presented in Table 5.

Table 5. Contraindications for intra-arterial thrombolytic treatment. Table modified based on Earnshaw J. (2010).

Absolute contraindications	Relative contraindications
Recent stroke or neurosurgery within 2 months	Major surgery/trauma within 10 days
Major surgery within 2 weeks	Hypertension >180 mmHg and diastolic >110 mmHg
Active bleeding disorder	Cardiopulmonary resuscitation within 10 days
Gastrointestinal bleeding within 10 days	Puncture of non-compressible vessel
Intracranial or spinal surgery within 3 months	Intracranial tumour
Head injury within 3 months	Pregnancy
	Recent eye surgery
	Diabetic haemorrhagic retinopathy
	Hepatic failure
	Bacterial endocarditis

### 2.6.4 Technical aspects of fibrinolytic therapy

Thrombolytic treatment for ALLI is mostly administered intra-arterially. In a Cochrane review, Kessel and colleagues (Kessel et al. 2004) demonstrated that the intravenous administration of thrombolytics was less effective than intra-arterial delivery directly into the thrombus. In fact, the total dose of lytics in the case of intra-thrombotic administration was obviously lower but simultaneously offered higher local drug concentrations.

Recently, new technical improvements in lytic therapy have been introduced. The data available on the results of such treatment are, however, limited. A Dutch randomized trial (Schrijver et al. 2015) compared CDT to ultrasound-accelerated thrombolysis. The rates

of technical success, severe adverse events and 30-day patency were comparable between the two methods. Ultrasound-accelerated thrombolysis, however, took significantly less time to achieve limb perfusion (Schrijver et al. 2015). High-dose thrombolysis has been suggested to offer significantly shorter treatment time without compromising the outcome in comparison to the standard-protocol CDT, as reported by Braithwaite and colleagues (1997). The more recent study by Plate and co-authors (Plate et al. 2006) prospectively evaluated high-dose pulse-spray with low-dose infusion alone and found no significant differences in regard to technical success and complications. The same authors found the re-interventions to be more frequent in patients treated with low-dose thrombolysis (Plate et al. 2006).

The standard CDT is performed through a puncture in the contralateral groin (Figure 9) or, occasionally, using an ipsilateral access. Local anaesthesia is used. The infusion of tPA is run through a multihole infusion catheter (e.g. a Cragg McNamara catheter [Medtronic, Minneapolis, Minn]) for a maximum of 48 hours. The current thrombolytic protocols are heterogenic in terms of the tPA dosage and treatment duration (Ebben et al. 2019). According to the same authors, lower doses of fibrinolytics lead to similar results at the cost of a longer treatment time but with a lower rate of bleeding complications.

Heparin or low-molecular-weight heparins are administered alongside CDT to stop the propagation of thrombosis, to prevent new embolic events and to maintain collateral circulation, as described in the previous chapter. A Swedish study group suggested that similar revascularization rates could actually be achieved in patients receiving no heparin (Grip et al. 2014). In fact, the bleeding complication rates of those patients were comparable to the bleeding rates of the individuals treated with heparin infusions (Grip et al. 2014).



Figure 9. The Cragg McNamara infusion catheter introduced “up and over” to the left side through the contralateral femoral artery. The insertion site in the groin is secured with a large adhesive bandage, which simultaneously compresses the puncture site and helps prevent “oozing bleeding”. ©Damir Vakhitov

## 2.6.5 Complications of thrombolysis

Haemorrhage, distal embolization and the development of compartment syndrome are the most common complications of CDT. In a review, which included five trials and 1,283 patients (Berridge et al. 2013), the risk of bleeding was more likely in thrombolysis patients (8.8%) compared to surgery patients (3.3%). The trend was similar in regard to distal embolization (12.4% versus 0%, respectively) within the same 30-day period (Berridge et al. 2013). It is of note that, in a recent Cochrane review, the evidence regarding an increased risk of major haemorrhage and distal embolization in thrombolysis patients was of low and very low quality, respectively (Darwood et al. 2018). The most dangerous haemorrhagic complication is, however, stroke, which occurs in approximately 1.3% of the patients (Berridge et al. 2013) and may lead to death.

The information available in regard to compartment syndrome (CS) development during treatment with CDT is insufficient (Figure 10). Although sparsely reported (Freyer et al. 2014), this serious complication usually occurs in up to 10% of the patients (Karnabatidis et al. 2011) and may lead to an increased risk of amputation and death (Eliason et al. 2003). The systemic consequences of CS are well recognized. Reperfusion oedema and excessive pressure inside the compartment may lead to tissue necrosis. The cell impairment results in the release of lactate dehydrogenase, intracellular potassium, creatine phosphokinase, phosphate, myoglobin and aldolase into the circulation (Petejova & Martinek 2014). The development of rhabdomyolysis may subsequently lead to renal failure. When CS is suspected, urgent actions must be taken. This includes four-compartment fasciotomy (Figure 11) either in a bilateral or a unilateral manner. Crystalloid fluids are administered to clear myoglobin, and forced diuresis is maintained. Placing the leg in an elevated position, as well as physiotherapy, rubber-band-assisted skin traction with a consequent wound closure (Figure 11) and negative-pressure wound treatment can reduce post-fasciotomy oedema. Nevertheless, free skin grafting may occasionally be required.

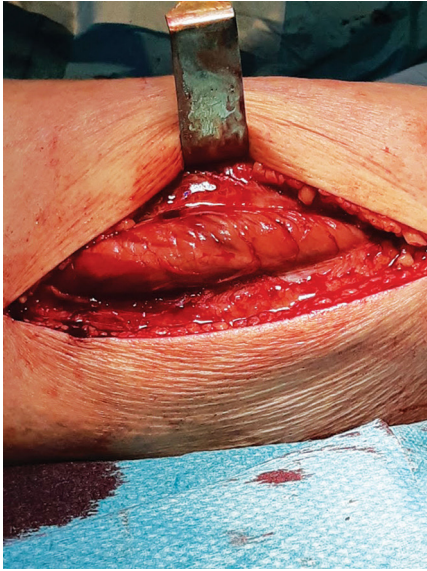


Figure 10. Compartment syndrome. An oedemic muscle steps out of the lateral compartment after the fascia has been opened. ©Damir Vakhitov



Figure 11. Bilateral four-compartment fasciotomy. The fasciae are opened from the condyli level and continued subcutaneously down to the ankle. The wounds are left open and rubber bands attached to the skin edges for traction and subsequent wound closure with staples or sutures. ©Damir Vakhitov. (The patient has recovered and can walk.)

### 2.6.6 Prognosis

Most studies have focused on the short- or mid-term results of thrombolytic treatment. The available information on the long-term outcome is insufficient and difficult to compare due to heterogeneity.

Despite the increasing use of newer mini-invasive endovascular techniques, the short-term amputation rates remain high and range from 10% to 30% (Byrne et al. 2014; Norgren et al. 2007). In a prospective population-based study from England that assessed

the outcome of all acute arterial peripheral events, over 70% of patients were either dead or dependent at the 6-month follow-up. The 5-year outcome was even more unfavourable, with almost 90% dead or dependent (Howard et al. 2015). In a large retrospective cohort study (Grip et al. 2017) that included 689 cases, the overall primary patency rate was 55.9% at 5 years, demonstrating superior results after embolic events (83.3%) over occluded stents/grafts (43.3%). An overall amputation-free survival rate of 45.2% at 5 years was registered, being lowest (37.9%) with occluded stents/grafts (Grip et al. 2017).

### 3 Aims of the Study

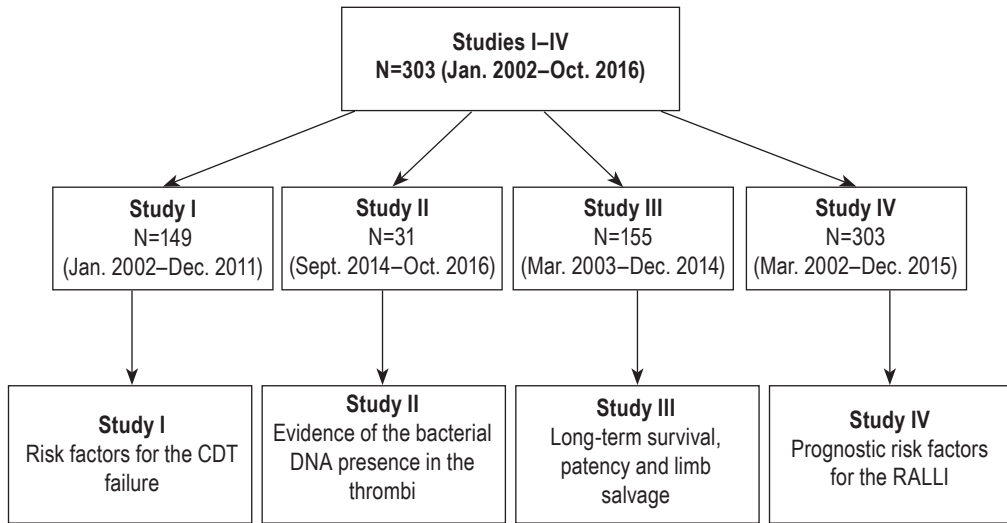
1. To study whether signs of bacteria are present in the thrombi of patients with ALLI.
2. To study the reasons of early thrombolytic treatment failure.
3. To assess the long-term outcomes of patients with ALLI after the initial treatment with CDT.
4. To assess the determinants of recurrent ALLI in patients initially treated with CDT.

## 4 Materials and Methods

### 4.1 Study population

This thesis is based on four original papers. They comprise one prospective study, two observational retrospective single-centre studies and one observational retrospective two-centre study (Table 6). The studies were performed at Tampere and Turku University Hospitals and Tampere University. The retrospective studies I, III and IV were based on prospective registries. Their data had been audited and additional information obtained from electronic case records and angiograms. The post-procedural angiograms in Study IV were evaluated to estimate the tibial runoff sufficiency by means of the crural index (Jalkanen et al. 2016; Wickström et al. 2017). Study II was based on prospectively collected information from patients with ALLI. Their thrombus aspirates were analysed in the laboratory of Tampere University by means of the quantitative polymerase chain reaction (qPCR). The results regarding the presence of bacterial DNA in the thrombi were confidentially transferred to the study group of vascular surgeons. The Ethics Committees of Tampere University Hospital and Turku University Hospital approved the studies. Informed consent was not required for Studies I, III and IV, while all patients gave their informed consent in Study II, in accordance with the study design and the instructions by the Ethics Committees. The dates and causes of death were obtained from electronic case records and from Statistics Finland.

Table 6. Summary of the numbers of studied patients and the main focus of each study



## 4.2 Methods

The study population comprises consecutive patients who were referred to Tampere and Turku University Hospitals. The studied patients presented with symptoms and signs of acute or acute-on-chronic lower limb ischaemia (Rutherford I–IIb). They were admitted to the hospital between March 2002 and December 2015. The patients were examined in the emergency room, with laboratory tests and radiological (ultrasonography, CT or MRI) examinations performed as necessary. Patients with contraindications for thrombolysis or severe sensorimotor loss at presentation were managed with open vascular surgical procedures or, in some cases, amputation. These patients are not presented in the current work. Patients unfit for either of the invasive treatment modalities were treated conservatively and excluded from further analysis.

### 4.2.1 Indications for treatment

The indications for treatment were the same in all the studies performed. They were based on the general symptoms that may be specific for ALLI. These often include acutely presented rest pain, claudication, cold sensation, discolouration, numbness and a motor deficit of the toes, foot or leg. If confirmed by the results of a clinical examination and, upon necessity, supported by radiological evidence, the indication for the treatment appeared clear.



### 4.2.2 Thrombolytic procedure

Interventional radiologists performed the majority of the procedures. The technical aspects of the thrombolytic treatment were partially described in section 2.5.3. The procedures were carried out through the common femoral artery under local anaesthesia. A contralateral approach was preferred. The thrombus aspirates in Study II were obtained aseptically in the angiography laboratory before the initiation of thrombolysis. Otherwise, an infusion of tPA (Actilyse; Boehringer Ingelheim, Stockholm, Sweden) was instantly started once the Cragg-McNamara infusion catheter (Medtronic, Minneapolis, Minn.) was inserted inside the thrombotic mass. An initial bolus of 4 mg was given. The treatment continued with a 0.5mg/h infusion for a maximum of two days, with the majority of control arteriographies performed every 24 hours.

The end point of the CDT was complete flow restoration. The thrombolytic therapy continued in a 24-hour observation ward or an intensive care unit until the fibrinolysis was completed. Additional endovascular or open surgical modalities could be needed to achieve adequate distal perfusion.

### 4.2.3 Follow-up

The patients were followed-up for various periods of time. After discharge from the hospital, the first follow-up visit was routinely scheduled at one month post-procedurally. Further surveillance was arranged at different time points until the patient required no additional manipulations and was free of all signs and symptoms of critical limb ischaemia. The patients who were treated for proximal bypass graft or native arterial occlusions underwent further annual examinations at their community health centres. Symptomatic patients underwent additional examinations. The patients who underwent below-the-knee bypass surgery were followed up biannually at the hospital. Their toe pressure and ankle-brachial index were assessed at each visit, along with ultrasound examinations. Additional visits were arranged upon necessity. These were based on the clinical situation.

### 4.2.4 Examination of thrombus aspirates for bacterial DNA presence

Study II evaluated the thrombi for bacterial DNA presence: any bacterial DNA and DNA of the *S. mitis* group. This group of bacteria comprises species most of which are from the oral cavity. These bacteria were found in thrombus aspirates of the coronaries and cerebral arterial wall samples in patients with acute coronary syndrome and cerebral aneurysms, respectively. The bacteria are known for their exceptional ability to bind platelets and to adhere to an endothelium, possibly suggesting its haematogenous means of spreading within the arterial flow.

In brief, the presence of microbial DNA was discovered with qPCR. The patients' own arterial blood served as a reference. The measurements were performed as duplicates or, in uncertain cases, as quadruples. The samples were marked bacterial-DNA-positive if there was an at least twofold difference in the relative amount of microbial DNA in comparison to the reference.

#### 4.2.5 Statistical analysis

IBM SPSS Statistics (IBM Corp. Armonk, NY) software was used to perform the statistical analyses. Fisher's exact test and Chi-squared tests were used to compare categorical variables. The Mann-Whitney  $U$  test and the independent samples  $t$ -test were applied for continuous variables. In Study III, age was, for descriptive purposes, divided into age categories ( $\leq 64$ , 65–74, 75–82.5 and  $\geq 83$  years). Univariable analysis was carried out using a Cox proportional hazards model. The variables were tested for a proportional hazards assumption with a log-minus-log plot. In Study I, all parameters were tested in a backward stepwise binary logistic regression model. In Studies III and IV, only the significant parameters were tested in a multivariable Cox backward stepwise regression model. Kaplan-Meier analysis was used for the patency and survival analyses. A  $P$ -value  $< 0.05$  for Studies I, III and IV was considered significant. Due to the relatively small size of the cohort in Study II, the statistical significance level was set to  $\leq 0.05$  instead.

## 5 Results

### 5.1 Bacterial DNA presence in thrombus aspirates obtained before the initiation of thrombolysis (Study II)

A total of 31 arterial thrombus aspirates were obtained from patients with ALLI (Table 7), including thrombi from native arteries as well as bypass grafts (Table 8). Of all the positive arterial samples 18/20 (90%) contained DNA of the *S. mitis* group. The mean difference in the amount of any bacterial DNA (total bacterial DNA) in comparison with the reference was 12.1 (median 7.1). For the *S. mitis* group DNA, the mean difference was 29.1, while the median was 5.2-fold. The only factor that was associated with the presence of DNA of the *S. mitis* group in the thrombi was a history of previous arterial interventions,  $p=0.049$ .

Table 7. Baseline demographics of patients in Studies I–IV. N/A – non-applicable

Baseline demographics	Study I N=149	Study II N=31	Study III N=155	Study IV N=303
Age, mean or median	70	71	73	72
Sex, female N(%)	70 (47.0)	13 (41.9)	82 (52.9)	144 (47.5)
Diabetes, N (%)	26 (17.4)	10 (32.2)	34 (21.9)	74 (24.4)
Dyslipidaemia, N (%)	70 (47.0)	17 (54.8)	78 (50.3)	116 (38.3)
Arterial hypertension, N (%)	120 (80.5)	25 (80.6)	120 (77.4)	223 (73.6)
Coronary heart disease, N (%)	66 (44.3)	6 (19.4)	69 (44.5)	117 (38.6)
Cerebrovascular disease, N (%)	19 (12.8)	3 (9.7)	21 (13.5)	43 (14.2)
Pulmonary insufficiency, N (%)a	N/A	7 (22.6)	22 (14.2)	51 (16.8)
History of smoking within the last 5 years, N (%)	41 (27.7)	15 (48.4)	26 (16.8)	65 (21.5)
Renal insufficiency, N (%)	7 (4.7)	2 (6.5)	15 (9.7)	19 (6.3)
Atrial fibrillation, N (%)	60 (40.3)	10 (32.3)	70 (45.2)	117 (38.6)
Heart failure, N (%)	25 (16.8)	4 (12.9)	37 (23.9)	62 (20.5)
Duration of ischaemia, days, median	3	7	4.7	2
Ischaemia category, Rutherford, N (%)				
Category I	85 (57.0)	N/A	57 (36.8)	59 (19.5)
Category IIa	50 (33.6)	N/A	98 (63.2)	130 (43.0)
Category IIb	14 (9.4)	N/A	N/A	114 (37.6)
Proximal anatomic segment involved, N (%)				
iliac	N/A	N/A	15 (9.7)	38 (12.5)
fem-pop	N/A	N/A	11 (7.1)	217 (71.4)
tibial	N/A	N/A	31 (20.0)	48 (15.8)

Table 8. The presence of bacterial DNA in native arterial and arterial bypass graft thrombi. Percentage presented within the subgroups of native arteries and conduits.

	All patients (N=31)	Positive total bacterial DNA (N=20)	Positive for <i>S. mitis</i> group DNA (N=18)
Type of thrombus aspirate, N (%):			
Native artery	19 (61.3)	11 (57.9)	10 (52.6)
Bypass graft:			
Autologous vein graft	3 (9.7)	2 (66.7)	2 (66.7)
Prosthetic conduit	9 (29.0)	7 (77.8)	6 (66.7)

## 5.2 Short-term outcomes (Studies I, II, III and IV).

Among the 303 ALLI cases (Table 7) managed with CDT, nearly 40% of the events in the native arteries were classified as embolism. Bypass graft occlusions were chiefly of thrombotic origin. The embolic events were associated with a history of atrial fibrillation.

Nearly a third of the cases were managed with thrombolysis as a monotherapy. The majority (>50%) required endovascular, open or hybrid procedures as an adjunct in order to achieve adequate distal perfusion. A total of 23% of the events were classified as a treatment failure (Table 9). Periprocedural complications are summarized in Table 10.

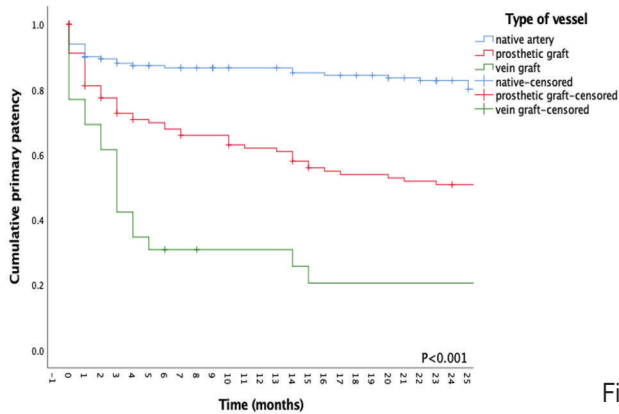
The analysis of primary patency is presented in Figure 12. The post-procedural primary patency rates of native arteries were significantly better than those of bypass grafts.

Table 9. Thrombolytic treatment failure criteria and incidence rates

<b>Failure criteria</b>	<b>Frequency, N (%)</b>
Rapid progression of ischaemia requiring conversion to open surgery	4 (2.7)
No lysis effect within two days of treatment	10 (6.7)
Complete or partial lysis with inability to reopen run-off vessels	10 (6.7)
Reocclusion within one month of thrombolysis	5 (3.4)
Major amputation above the ankle level due to ischaemia	13 (8.7)
Termination of thrombolysis due to major bleeding	2 (1.3)
In-hospital death	4 (2.7)

Table 10. Periprocedural complication rates

<b>Complication types</b>	<b>(%)</b>
Major bleeding	6
Compartment syndrome	1.3
Intracranial bleeding and death	0.7



	No and percent at risk				
(0 point)	1 month	6 months	12 months	18 months	24 months
Native artery - 164	134 (90%)	122 (87%)	114 (87%)	107 (84%)	95 (83%)
Prosthetic - 113	87 (81%)	70 (68%)	62 (62%)	52 (54%)	48 (51%)
Vein graft - 26	18 (69%)	7 (31%)	6 (31%)	4 (21%)	4 (21%)

Figure 12. Primary patency of native arteries and bypass grafts. Kaplan-Meier survival analysis,  $p < 0.001$ . Limbs at risk and percentage (in brackets) presented in the table.

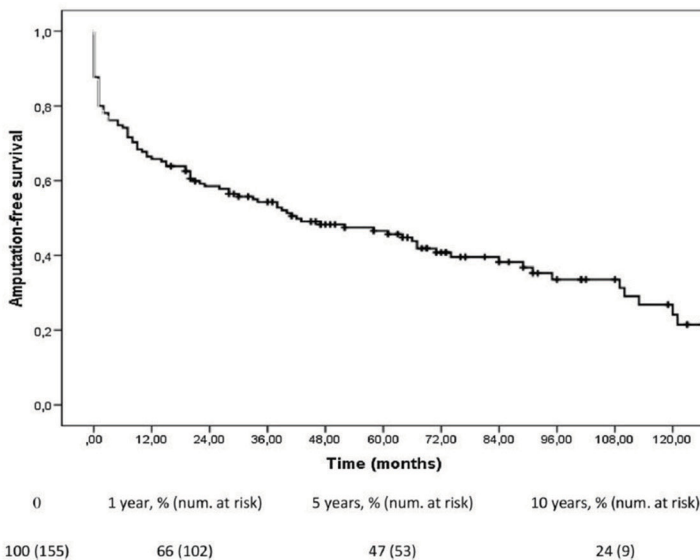


Figure 13. Amputation-free survival after CDT for ALLI. Kaplan-Meier survival analysis.

Almost 9% ( $N=13/149$ ) of the major amputations were performed within the first month after thrombolysis due to a failure to restore sufficient circulation in the limb ( $N=13/34$  [38%] within the failure group). Approximately 80% of the patients were alive at one year post-procedurally, with the amputation-free survival rate exceeding 60% (Figure 13).

## 5.2.1 Risk factors associated with treatment failure within a 30-day period

Previous bypass grafting (odds ratio [OR] 0.18), dyslipidaemia (OR 0.16) and a delay in treatment initiation (OR 0.95 per day) negatively impacted on the success of thrombolysis in the binary logistic regression analysis. The trend was present throughout the post-procedural follow-up when evaluated by Cox Regression analysis; see Figures 14–18.

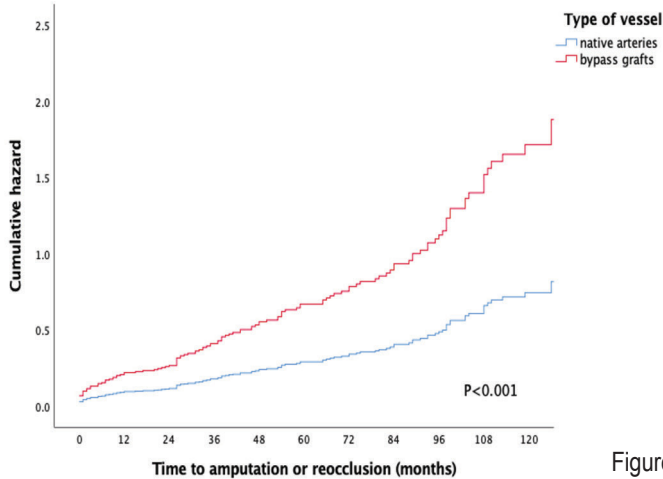
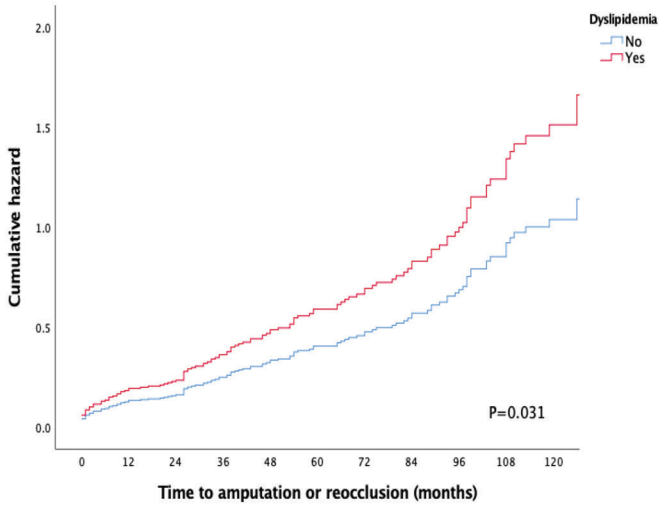


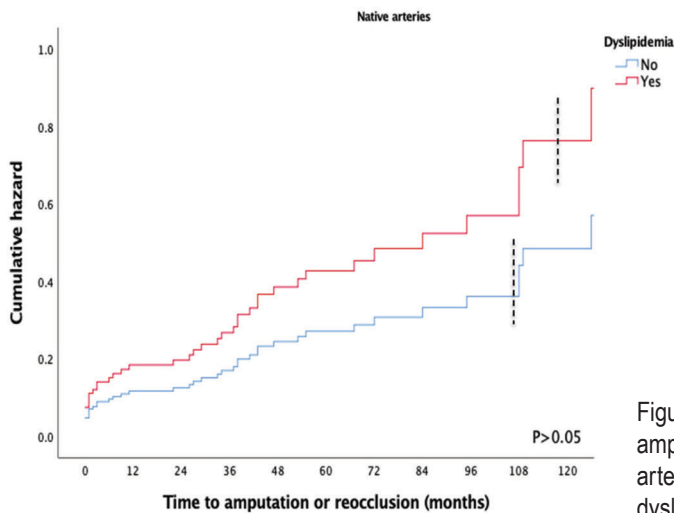
Figure 14. Cumulative hazard of amputation or reocclusion in patients with native arterial and bypass graft events. Cox Regression analysis,  $p < 0.001$ . Limbs at risk presented in the table.

(0 point)	No at risk				
	1 year	3 years	5 years	7 years	9 years
Native artery - 164	111	81	51	33	20
Bypass graft - 139	110	77	52	34	15



(0 point)	No at risk				
	1 year	3 years	5 years	7 years	9 years
No dyslipidemia - 187	142	95	67	46	23
Dyslipidemia - 116	78	63	36	21	12

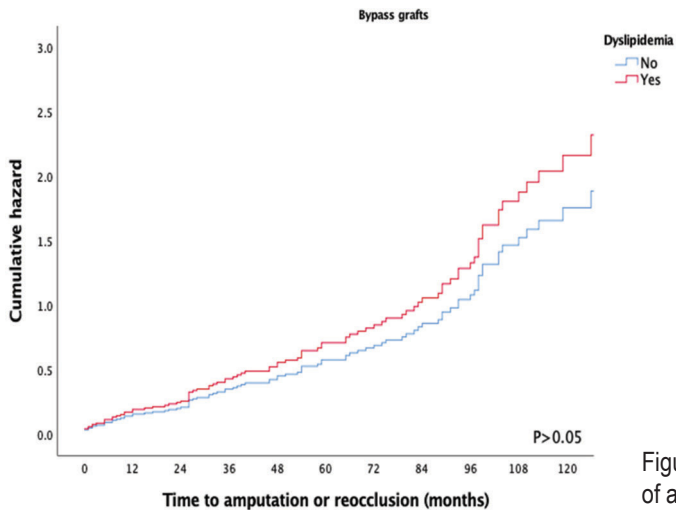
Figure 15. Cumulative hazard of amputation or reocclusion in patients with and without dyslipidaemia. Cox Regression analysis,  $p=0.031$ . Limbs at risk presented in the table.



(0 point)	No at risk				
	1 year	3 years	5 years	7 years	9 years
No dyslipidemia - 109	78	54	34	24	15
Dyslipidemia - 55	33	28	17	9	6

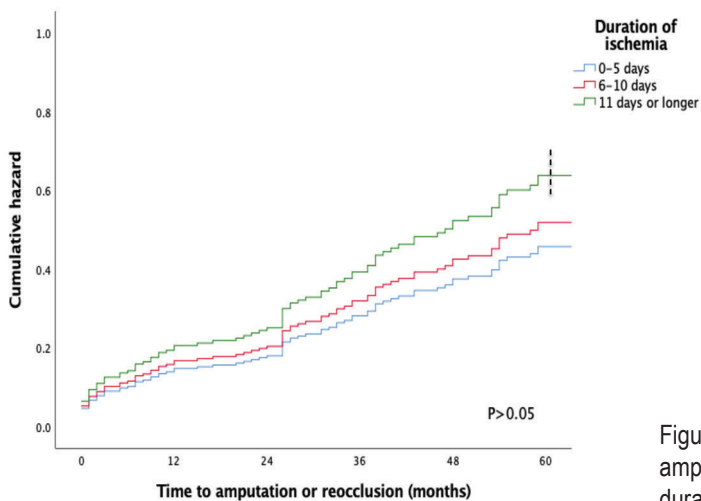
Figure 16. Cumulative hazard of amputation or reocclusion in native arterial patients with and without dyslipidaemia. Cox Regression analysis,  $p>0.05$ . The vertical dashed line represents the level at which the standard error exceeds 10%. Limbs at risk presented in the table.





(0 point)	No at risk				
	1 year	3 years	5 years	7 years	9 years
No dyslipidemia - 78	64	42	33	22	9
Dyslipidemia - 61	46	35	19	12	6

Figure 17. Cumulative hazard of amputation or reocclusion in bypass graft patients with and without dyslipidaemia. Cox Regression analysis,  $p > 0.05$ . Limbs at risk presented in the table.

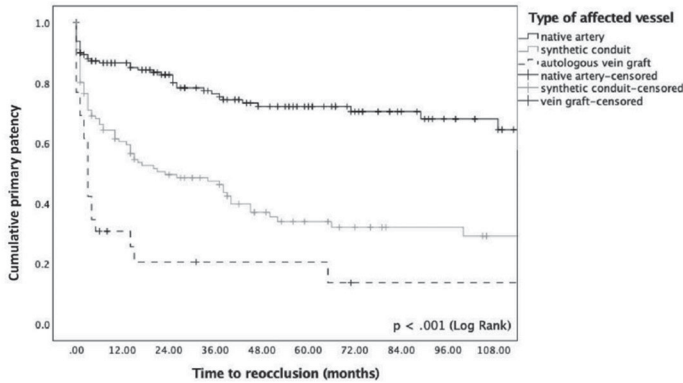


(0 point)	No at risk		
	1 year	3 years	5 years
0-5 days - 209	157	117	56
6-10 days - 37	23	19	11
$\geq 11$ days - 43	30	15	7

Figure 18. Cumulative hazard of amputation or reocclusion by the duration of ischaemia symptoms. Cox Regression analysis,  $p > 0.05$ . The vertical dashed line represents the level at which the standard error exceeds 10%. Limbs at risk presented in the table.

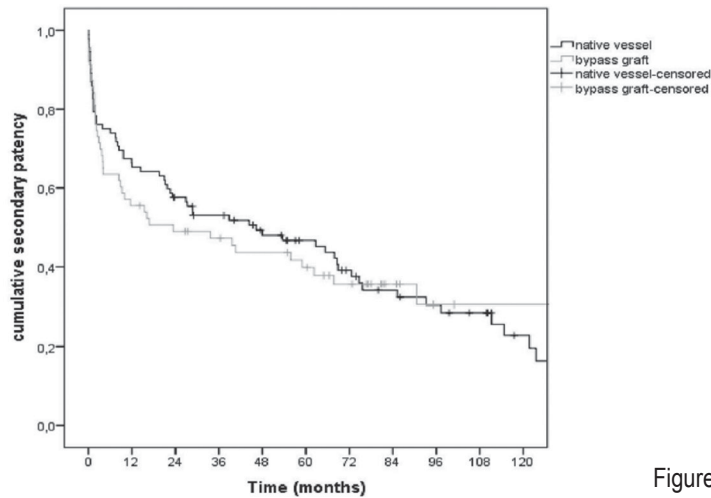
### 5.3 Long-term outcomes (Studies III and IV)

Almost 80% of the patients underwent additional open, endovascular or hybrid procedures to preserve sufficient limb circulation. The long-term outcomes were significantly different between the patients with native artery and bypass graft occlusions. The primary patency rates after native-arterial events were significantly more favourable than the corresponding rates after bypass graft occlusions and, in particular, after autologous vein bypass graft thrombosis. The latter demonstrated the lowest primary patency rates at 9 years post-procedurally (Figure 19). The long-term secondary patency rates showed an insignificant difference between native arteries and bypass grafts (Figure 20).



Limbs at risk:	1 year	3 years	5 years	7 years	9 years
Native artery N=164	114 (86.6)	78 (76.4)	51 (72.2)	30 (70.5)	19 (68.0)
Synthetic conduit N=113	62 (60.5)	41 (47.3)	19 (34.0)	11 (32.1)	8 (29.2)
Vein graft N=26	6 (30.8)	4 (20.5)	3 (20.5)	1 (13.7)	1 (13.7)

Figure 19. Cumulative primary patency. Kaplan-Meier survival analysis,  $p < 0.001$ . Limbs at risk and percentage (in brackets) presented in the table.



	1 year, % (num. at risk)	5 years, % (num. at risk)	10 years, % (num. at risk)
Native artery, N=92	65.2 (60)	46.7 (31)	22.8 (7)
Bypass graft, N=63	55.6 (35)	39.8 (21)	30.5 (4)

Figure 20. Cumulative secondary patency. Kaplan-Meier survival analysis,  $p=0.88$ . Limbs at risk and percentage (in brackets) presented in the table.

Predominantly bypass conduits were affected with the recurrent ischaemia, which, overall, occurred in over 40% of the cases within a median of 40 months.

Approximately 25% of the patients required major amputations during the follow-up. The amputation-free survival rate was 47% at 5 years and 24% at 10 years post-procedurally (Figure 13). The presence of rethrombosed prosthetic conduits was associated with superior limb salvage when compared to native arterial and vein graft thrombotic events,  $P=0.025$ . The most common cause of death was cardiovascular events (Figure 21). The cumulative survival of patients was 56% at 5 years and <30% at 10 years (Figure 22).

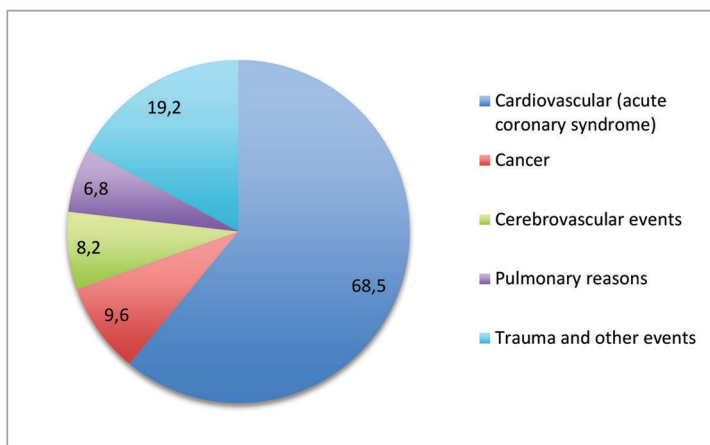


Figure 21. Distribution of causes of death in ALLI patients initially treated with CDT. Percentage provided.

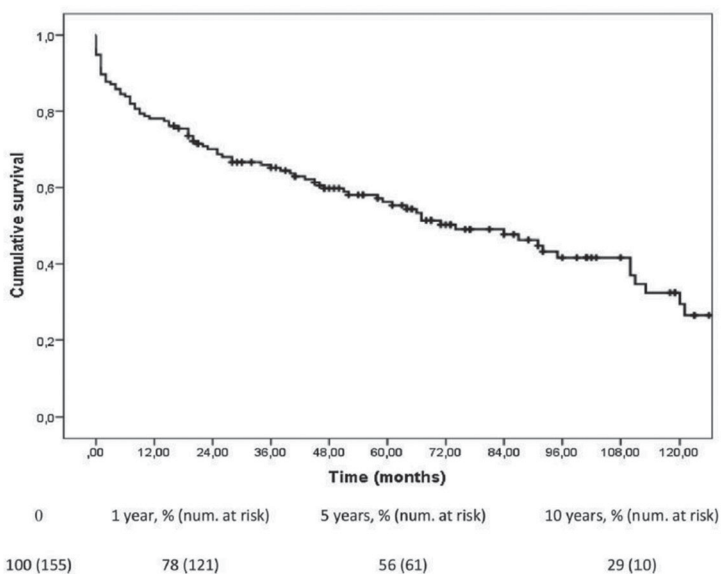


Figure 22. Cumulative survival of patients after treatment with CDT. Kaplan-Meier survival analysis.

### 5.3.1 Independent risk factors negatively affecting long-term outcomes

#### *Patency*

Decreased primary patency rates were associated with the absence of antiplatelet and/or anticoagulant treatment after native arterial occlusions (hazard ratio [HR] 6.51),  $p < 0.001$ . An increased risk of bypass graft rethrombosis was associated with diminished tibial runoff (HR 2.40),  $p < 0.05$ .

### *Major amputations*

The occurrence of a bypass graft thrombosis (HR 14.77),  $p=0.01$ , was associated with an increased risk of major amputations.

### *Amputation-free survival*

Age over 75 years was associated with diminished amputation-free survival rates (HR 2.01 per age category),  $p=0.04$ .

### *Cumulative survival*

Age over 83 years (HR 5.23 per age category),  $p<0.001$ , and atrial fibrillation (HR 2.31),  $p=0.001$ , were associated with diminished cumulative survival rates.

## 6 Discussion

The current work focuses on the possible aetiological aspects of thrombosis and the long-term outcome of thrombolytic treatment in patients with ALLI. Tampere and Turku University Hospitals serve as referral centres to a combined population of almost 2 million people; therefore, the data presented particularly in Study IV is among the largest available in the literature.

### 6.1 Evidence of bacterial DNA presence in thrombus aspirates of patients with ALLI

Our study revealed the presence of microbial DNA in the thrombus aspirates of patients with symptomatic arterial thrombosis of the lower extremities. DNA of the *S. mitis* group was predominantly presented in the thrombi.

Previous studies (Pessi et al. 2013; Pyysalo et al. 2013, 2016) have found DNA of the *S. mitis* group to be the most common in thrombus aspirates from cerebral arterial wall samples and coronaries in patients with cerebral aneurysms and acute coronary events, respectively. Oral bacteria can access the arterial flow and reach the “destination site” via lesions in the oral cavity. Furthermore, micro-organisms can access atherosclerotic plaques from the vasa vasorum. These small vessels surround the arterial adventitia and provide nutrient supply. The bacteria of the *S. mitis* group have been shown to be able to bind thrombocytes (Bensing et al. 2001; Mitchell et al. 2007). The bacteria of this group can create thrombogenic and inflammatory biofilms after adhering to an endothelium. This allows other micro-organisms to attach as well (Kolenbrander et al. 2010). Bacteraemia may result in an accumulation of microbes in the plaque, which may lead to the subsequent development of a low-grade inflammation (Pessi et al. 2013). The rupture of a plaque inhabited by micro-organisms with a thrombocyte-binding potential may lead to the formation of a thrombus. This supports the potential role of such micro-organisms in

vascular thrombosis, which usually requires endothelial injury and the presence of platelets (Yeaman 2010). Bacteria of the oral cavity have been found in the endocardium, heart valves or atherosclerotic plaques (Kerrigan & Cox 2009).

Although the results of our study are interesting, the information must be interpreted with caution. Currently, the role of bacteria in the setting of thrombosis is not understood completely, and there are some drawbacks in the study. The revealed “bacterial signature” in the thrombi of ALLI patients is, however, the first step towards understanding the mechanisms of possible bacterial involvement in the pathophysiology of thrombosis.

## 6.2 Prognostic determinants of short-term outcome after treatment with thrombolysis

A success rate of 77% was registered in Study I, and it is in accordance with the results of the available data from other centres (Kuoppala et al. 2008; Plate et al. 2009). Early failure was found to be associated with the presence of hypercholesterolaemia, prolonged ischaemia duration and previous bypass grafting. Attention must be paid to the first two determinants, as the last one will be discussed in the next section.

The role of dyslipidaemia in the setting of ALLI has not been sufficiently studied. There is no strong data to support or reject the direct role of hypercholesterolaemia in lower-limb thrombotic or embolic events. There is, however, data to propose that dyslipidaemia may impair the endothelium-related relaxation and increase the vascular tone, in addition to decreasing the blood flow and increasing platelet aggregation (Komori et al. 1989). Such mechanisms may predispose patients to the occurrence of PAD, as reported by the same authors. Furthermore, this data can be supported by the fact that, in the Reduction of Atherothrombosis for Continued Health registry, statin therapy was associated with a 17% decrease in adverse cardiovascular event rates (Kumbhani et al. 2014). In our retrospective study, we could not demonstrate the prevalence of statin users and the lipid levels among the patients of the cohort. Nonetheless, based on the evolving data, we are prone to believe that statin treatment is important. In the recent European Society for Vascular and Endovascular Surgery guidelines released in March 2018, all patients with PAD are suggested to be prescribed statin therapy (Aboyans et al. 2018), thus supporting our findings.

A delay in treatment initiation has been suggested to negatively impact on thrombolysis results (Working Party on Thrombolysis in the Management of Limb Ischemia 2003). The results of the STILE trial suggested that patients with acute symptoms lasting no longer than two weeks could be treated with thrombolysis (The Stile Investigators 1994). In our series, the difference between the success and failure groups in regard to the median duration of symptoms was insignificant (Study I). Nevertheless, in the logistic regression analysis, a prolonged duration of ischaemia was independently associated with an increased

risk of treatment failure by 5% per day. It is of note that the duration of symptoms is often difficult to evaluate exactly. This measure may be dependent on the individual differences in experiencing a sensory deficit and pain.

## 6.3 Long-term outcomes of patients with ALLI after initial treatment with CDT

### 6.3.1 Patency

Despite the large number of additional procedures needed to preserve adequate distal perfusion, the results of our studies suggest that the long-term patency rates after the initial treatment with CDT are generally unfavourable. This is particularly true for bypass conduits, the primary patency rates of which drop to below 30% at 9 years post-procedurally. The primary patency rates after native arterial occlusions are higher, but they are dependent on the patients' baseline characteristics and the presence of post-procedural medication.

#### *6.3.1.1 Native arteries*

Our analysis has demonstrated that the absence of proper antiplatelet or anticoagulant therapy after native arterial occlusions is independently associated with recurrent ALLI (RALLI) events. While nearly 40% of native arterial occlusions were of embolic origin, they were significantly associated with the history of atrial fibrillation. Previous studies (Campbell & Ridler 2000; Forbes et al. 2002) suggested the importance of anticoagulation in the prevention of RALLI. This is particularly true for those in whom the condition develops due to cardiac reasons (Forbes et al. 2002). There is, however, insufficient data available to reject or support the long-term use of anticoagulants in patients with no intracardiac thrombus or atrial fibrillation (Forbes et al. 2002). The efficacy of acetylsalicylic acid (ASA) has been documented in a large number of studies (Eikelboom et al. 2012). The use of dual antiplatelet therapy appears to be promising in reducing post-procedural complications in patients with PAD (Beiswenger et al. 1922; Katsanos et al. 2015). Additionally, the data from a recent trial proposed that a combination of aspirin with rivaroxaban for secondary cardiovascular prevention was superior to ASA alone or rivaroxaban alone (Eikelboom et al. 2017). Although we could not draw final conclusions on the regime for post-procedural medication in the current study, it is clear that the absence of such treatment could be decisive.

#### *6.3.1.2 Bypass grafts*

More advanced stages of PAD are frequent among the cases of bypass graft occlusions. The progressing PAD may predispose these patients to poorer results in comparison to patients with native arterial events. Vein conduits are usually used to correct more distal



(below-knee), extensive lesions, whereas synthetic grafts are often used above the knee. Therefore, the latter group of patients is usually characterized with less-severe underlying PAD. Although CDT is generally an effective remedy in conduit recovery, the patency of such grafts is, nevertheless, poor (Chalmers et al. 1995). This phenomenon may be partially explained by the progression of atherosclerosis and a worsened outflow as a result. In our series, an impaired tibial outflow was an independent predictor of bypass graft reocclusions. Furthermore, the development of a prothrombotic layer in the prosthetic conduits and vein graft endothelial alterations could also lead to an unfavourable outcome (Conrad et al. 2003). According to another study, occluded vein grafts are unlikely to yield durable post-thrombolytic patency (Nackman et al. 1997). Although our results generally confirm the existing data (Grip et al. 2017; Kuoppala et al. 2008), a more precise comparison is difficult to achieve owing to the diverse selection of end points, study structures and methods used.

### 6.3.2 Major amputations and amputation-free survival

In our series, over a quarter of the ALLI patients underwent major amputations. The main reasons were RALLI and an inability to obtain sufficient distal perfusion. The increased risk of amputation was associated with the occurrence of graft thrombosis. The majority of amputations were performed within thirty days from the RALLI. The only independent parameter to be associated with unfavourable amputation-free survival was an age of over 75 years (Study III).

The amputation-free survival rates of patients with synthetic conduits were superior to the rates of those who had suffered native arterial events and autologous vein graft thrombosis events (Study III). This trend was also similar after RALLI (Study IV). The data available to address this issue is inadequate (Byrne et al. 2014; Grip et al. 2017; Genovese et al. 2016; Taha et al. 2015; Nackman et al. 1997) and difficult to compare to our results due to the heterogeneity of the works. According to our series, almost all of the patients with recurrent events in native arteries presented with considerably diminished outflow. The inability to re-obtain adequate circulation led to major amputations. Vein bypass grafts could be exposed to irreversible ischaemic changes in the endothelium, which resulted in poor patency rates. As described earlier, vein conduits are generally used in distal, below-the-knee bypass surgery. The inability to restore circulation in the limb, along with advancing distal atherosclerotic changes, would apparently predispose many of these patients to major amputations. Conversely, prosthetic grafts are frequently used in more proximal segments. Even though the patency of recovered conduits is generally poor, the extremity can be saved by means of additional surgical operations.

### 6.3.3 Cumulative survival

Poor short-, medium-, and long-term survival of elderly patients after acute ischaemic events of the lower extremities has been acknowledged lately (Grip et al. 2017; Koraen et al. 2011; Kuoppala et al. 2008; Schrijver et al. 2016; Taha et al. 2015). Increasing age has been shown to be significantly associated with higher mortality rates within five years from CDT (OR 1.07/year) (Grip et al. 2017). In our study, we confirmed these findings, identifying poor mid- and long-term post-lytic survival rates (HR 5.23/per age category). The majority of deaths resulted from cardiovascular causes, especially in octo- and nonagenarians. Age is, nevertheless, a non-modifiable, natural determinant. The association of advanced age with mortality is, therefore, understandable.

The influence of atrial fibrillation (AF) on the mortality of ALLI patients managed with thrombolysis is uncertain. Moreover, the existing data are conflicting. Nonetheless, the accumulating evidence supporting the role of AF in the increased mortality (Andersson et al. 2013) and the correlation with cardiovascular diseases (Odutayo et al. 2016) supports our findings. In the cohorts studied, the patients with atrial fibrillation were mainly prescribed warfarin. It should be emphasized that, due to the changing international normalized ratio and difficulties in its maintenance within a certain range, the efficacy of warfarin may be insufficient in some patients (Birman-Deych et al. 2006). It remains debatable whether newer anticoagulation alternatives could offer better protection and increase the survival rates of ALLI patients.

## 6.4 Limitations of the study

In our series, both prospective and retrospective cohorts were analysed. Study II is a prospective work, and it may be criticized for its relatively small number of samples. It was a “pioneer” study, in which it took our team some time to recruit the patients and process the samples. Nonetheless, the results are clear and the work opens good perspectives for future projects. We reported the presence of bacterial DNA. The study, however, did not reveal the presence of living micro-organisms or their influence on thrombosis. The problem was difficult to overcome because staining and culturing in our study would have had a rather limited potential. In contrast, the use of qPCR was accurate. We admit that the presence of microbial DNA in thrombi can also take place if the binding of bacteria with platelets occurs elsewhere in the circulatory system. Furthermore, inflammatory cells with eliminated microbial DNA fragments could also be attracted to the platelet aggregation site. However, the multi-fold differences in the volumes of DNA discovered in the thrombi suggest their possible role in the course of the thrombotic process. The function that bacteria serve and whether the function is important remain to be studied further.

Although based on a prospectively maintained registry, Studies I, III and IV are retrospective, which is a limitation. Nonetheless, they include relatively large numbers of

patients. Furthermore, the same investigators processed the data, which we hope could reduce the risk for errors. One final issue is the selection bias. The patients were selected and underwent thrombolytic treatment based on the clinical assessment of a vascular specialist.

## 6.5 Future perspectives

The aetiology and pathogenesis of ALLI has been gradually changing. The patients increasingly present with atherosclerosis-associated thrombosis rather than embolism. Currently, vascular surgeons deal with elderly patients who have multiple cardiovascular comorbidities. This often requires decision-making in a multidisciplinary group of specialists. Increasing obesity has also become an obstacle. According to the World Health Organization (2018), excess weight and obesity are now dramatically on the rise even in low- and middle-income countries and have become major risk factors for cancer, cardiovascular diseases and diabetes. In this setting, the role of open surgery as an initial treatment modality may face further recession, whereas minimally invasive, endovascular procedures will appear more appropriate particularly for the treatment of ALLI. The recent results of the Compass trial (Eikelboom et al. 2017) that suggest the use of rivaroxaban and aspirin as well as the increasing use of statins in PAD patients (Kumbhani et al. 2014) may have a positive impact on the outcome. Further competition between medical companies will probably reduce the costs of pharmacomechanical thrombolysis. The use of this method will permit reduced treatment times and decreases in overall expenses. It is, however, unlikely that the existing form of pharmacomechanical thrombolysis will completely replace CDT. The role of aspiration thrombectomy in ALLI treatment is possibly underestimated in some vascular centres and, in fact, remains unjustified. Furthermore, the research on the role of bacterial inflammation in the pathogenesis of thrombosis could possibly bring new therapeutic options in the long term.

The aforementioned aspects raise new questions that we have currently no answers to. Further investigations are needed not only from the clinical viewpoint but also from the economic stand. This will benefit patients, health care practitioners and society as a whole.

## 7 Conclusions

1. Bacterial DNA, particularly of the *S. mitis* group, is present in the thrombi of patients with ALLI. The presence of bacterial signs is significantly associated with a history of previous arterial interventions.
2. The majority of ALLI cases can be successfully managed with CDT. Patients presenting with hypercholesterolaemia, a prolonged duration of ischaemia and previous bypass grafting are at a higher risk of CDT failure in the short term.
3. Although multiple additional procedures are needed to preserve adequate distal perfusion after treatment with CDT, the long-term amputation-free survival rates are unfavourable.
4. Recurrent acute lower limb ischaemia occurs frequently, as over 40% of patients are exposed to this condition. Bypass grafts are at a higher risk of reocclusions. The absence of appropriate post-thrombolytic anticoagulant or antiplatelet treatment after native arterial events is independently associated with the occurrence of RALLI. A diminished outflow is independently associated with rethrombosis in bypass grafts.

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## Original publications



# PUBLICATION

I

**Independent factors predicting early lower limb intra-arterial thrombolysis failure**

Vakhitov D, Suominen V, Korhonen J, Oksala N, Salenius JP

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# Independent Factors Predicting Early Lower Limb Intra-arterial Thrombolysis Failure

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**Background:** Risk factors for early catheter-directed intra-arterial thrombolysis failure in acute lower limb ischemia remain unclear.

**Methods:** One hundred forty-nine limbs with acute artery or bypass graft thrombosis underwent catheter-directed thrombolysis (maximum of 48 hours). A retrospective data analysis was carried out to assess possible risk factors for early, 30-day treatment failure.

**Results:** Seventy-nine men (53%) and 70 women (47%) with a median age of 70 (range 32–93) years were treated. Treatment outcomes were determined as success ( $N = 115$ , 77%) or failure ( $N = 34$ , 23%). The failure criteria comprised rapid progression of ischemia ( $N = 4$ , 2.7%) and major bleeding complications ( $N = 2$ , 1.3%), both requiring thrombolysis termination and surgery. Inability to reopen native arteries/grfts ( $N = 10$ , 6.7%), run-off vessels ( $N = 10$ , 6.7%), in-hospital death ( $N = 4$ , 2.7%), the need for major amputation ( $N = 13$ , 8.7%), and reocclusions ( $N = 5$ , 3.4%) within the 30-day follow-up period were also considered as failures. Multivariate analysis of the risk factors' impact on the success of thrombolysis revealed such independent parameters as hypercholesterolemia (OR 0.16, 95% CI 0.06–0.42,  $P < 0.0001$ ), previous bypass grafting of the ipsilateral limb (OR 0.18, 95% CI 0.06–0.53,  $P = 0.002$ ), and duration of ischemia prior to the initiation of thrombolysis (OR 0.95, 95% CI 0.91–0.99,  $P = 0.009$ , per day).

**Conclusion:** According to our results, factors independently predicting early failure include hypercholesterolemia, previous bypass grafting, and a delay in treatment initiation. Moreover, catheter-directed intra-arterial thrombolysis can be considered safe and effective in the treatment of acute lower limb ischemia.

## INTRODUCTION

For more than 20 years, intra-arterial catheter-directed thrombolysis has been used widely for the treatment of lower limb acute ischemia. Previous results<sup>1–3</sup> suggest that the method itself is safe and effective, with a success rate of approximately

70–80%. Good run-off vessels and intrathrombotic infusion have been shown to be major determinants of a successful immediate outcome of thrombolytic therapy.<sup>4–6</sup> Severe stages of ischemia before thrombolysis initiation, on the other hand, are associated with an increased amputation rate.<sup>2,7,8</sup>

To date, a number of studies have analyzed the possible role of comorbid conditions in early and long-term outcome of lower limb intra-arterial thrombolysis.<sup>1,2,4,7–10</sup> According to the available literature, diabetes mellitus (DM) and coronary heart disease (CHD) are associated with poor post-procedural outcome.<sup>2,4,7,8</sup> Furthermore, some studies have demonstrated a distinct relationship between thrombolysis-related mortality and factors such as cerebrovascular disease, chronic renal insufficiency, female gender, and increasing age.<sup>2,7</sup> These results are not, however, consistent, as other investigators have suggested quite the contrary.<sup>1,11</sup>

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Patients with acute ischemia often have multiple comorbid conditions that may affect their long-term prognosis despite successful thrombolysis.<sup>2</sup> Moreover, the progression of underlying peripheral arterial disease over time can worsen the clinical picture. Consequently, in this retrospective, vascular registry-based study, we decided to focus particularly on the early outcome of thrombolytic therapy. Apart from the aforementioned studies, most of which concentrated on factors associated with major amputation and mortality after thrombolysis, we decided to look at the problem from another point of view, by trying to define the factors associated with the failure of thrombolysis itself. We hypothesized that certain cardiovascular risk factors and conditions have a direct impact on the early outcome.

## METHODS

### Patients

This study was a retrospective analysis of consecutive patients ( $N = 327$ ) referred to Tampere University Hospital (TAUH), Finland, for acute ischemia between January 2002 and December 2011. TAUH serves as a tertiary vascular surgical referral center for approximately 1.2 million people. Patients with symptoms or signs of category I–IIb acute limb ischemia<sup>12</sup> presenting with angiographic evidence of native artery or bypass graft thrombosis were included in the study on an intention-to-treat basis ( $N = 144$ ). During the study, 5 patients were referred twice. All these patients were treated with thrombolysis, and the actual study group thus comprised 149 limbs. Patients with progressive sensorimotor changes, clinically or radiologically identified embolic events, or contraindications for thrombolysis ( $N = 182$ ), as well as those in whom guide-wire or catheter introduction was, for any reason, unsuccessful ( $N = 1$ ), were treated surgically and excluded from further analysis.

### Thrombolysis Protocol and Follow-up

All procedures were performed under local anesthesia by an interventional radiologist. Percutaneous mechanical thrombectomy was not used in the cohort observed. The catheter was placed in the thrombotic mass and the recombinant tissue plasminogen activator alteplase (Actilyse<sup>®</sup>; Boehringer Ingelheim, Stockholm, Sweden) was administered as an initial 4-mg bolus, followed by a continuous 0.5-mg/hr infusion lasting for a maximum of 48 hr. At the same time, the patients were administered

low-molecular-weight heparins (LMWHs) enoxaparin sodium (Klexane<sup>®</sup>; Sanofi-Aventis, Maisons-Alfort, France) 40 mg twice per day subcutaneously or dalteparin sodium (Fragmin<sup>®</sup>; Pfizer, Puurs, Belgium) 5000 IU twice per day subcutaneously. The patients then remained in 24-hr observation with blood tests (full blood count, potassium, sodium, creatinine, creatine kinase, myoglobin) controlled at least twice daily. Angiography controls were performed every 24 hr. Necessary surgical or endovascular procedures (percutaneous angioplasty [PTA], or stenting) were performed upon completion of thrombolysis. In cases of successful monotherapy–thrombolysis, when blood clotting reason remained unclear, the patients underwent additional examinations (hematologic, cardiologic, internal medicine). Warfarin and/or combinations of other drugs were administered for various periods in these cases (data not shown). During the 30-day follow-up visit, clinical status was assessed using the ankle–brachial index, toe-pressure assessment, and an ultrasound examination if needed (data not shown).

### Comorbid Conditions

Data from patient files and the vascular registry were collected systematically by 1 examiner (D.V.). The following risk factors from case records were included in the analysis: age; gender; diabetes mellitus; hyperlipidemia; hypertension; heart failure; smoking within 5 years; cardiovascular diseases (peripheral artery disease [PAD], coronary heart disease [CHD], cerebrovascular disease); chronic renal failure; and severity of ischemia. The diagnosis for each disease was considered positive if it had been previously established at the hospital or mentioned in the referral, or if the patient was on appropriate medication. The diagnosis of PAD was based on vascular changes in a digital subtraction angiogram (DSA) and defined as a narrowing of the arterial lumen of >50% in any arterial segment of the lower limbs. In addition, information on the duration of acute symptoms, previous vascular interventions, and aneurysmal disease were searched for and included in the analysis.

### Outcome Measures

The treatment was considered successful (success group) if the native vessels or bypass grafts treated with thrombolytic therapy were patent upon the completion of thrombolysis and if acute symptoms had resolved and the achieved clinical status remained stable for 1 month. The need for additional minor surgical (endarterectomy, patch plasty,

**Table I.** Patients' characteristics (data shown as per limb treated)

Variables	Total ( <i>n</i> = 149)	Success ( <i>n</i> = 115)	Failure ( <i>n</i> = 34)	<i>P</i> -value
Median age <sup>a</sup> [ <i>n</i> (%)]	70 (32–93)	71 (32–93)	68 (50–93)	0.342
Gender, female <sup>b</sup> [ <i>n</i> (%)]	70 (47%)	50 (43.5%)	20 (58.8%)	0.115
Arterial hypertension <sup>b</sup> [ <i>n</i> (%)]	120 (80.5%)	91 (79.1%)	29 (85.3%)	0.425
Atrial fibrillation <sup>b</sup> [ <i>n</i> (%)]	60 (40.3%)	44 (38.3%)	16 (47.1%)	0.358
Heart failure <sup>b</sup> [ <i>n</i> (%)]	25 (16.8%)	20 (17.4%)	5 (14.7%)	0.713
Hypercholesterolemia <sup>b</sup> [ <i>n</i> (%)]	70 (47%)	43 (37.4%)	27 (79.4%)	<0.0001*
Diabetes <sup>b</sup> [ <i>n</i> (%)]	26 (17.4%)	18 (15.7%)	8 (23.5%)	0.288
Smoking <sup>b</sup> [ <i>n</i> (%)]	41 (27.7%)	28 (24.3%)	13 (39.4%)	0.089
Previous bypass grafting <sup>b</sup> [ <i>n</i> (%)]	76 (51%)	51 (44.3%)	25 (73.5%)	0.003*
Aneurysm proximally <sup>b</sup> [ <i>n</i> (%)]	7 (4.7%)	6 (5.2%)	1 (2.9%)	0.582
Previous vascular intervention within 1 month <sup>b</sup> [ <i>n</i> (%)]	13 (8.7%)	9 (7.8%)	4 (11.8%)	0.475
Duration of symptoms <sup>a</sup> [median number of days (range)]	3 (1–60)	3 (1–30)	2 (1–60)	0.962
Coronary disease <sup>b</sup>	66 (44.3)	47 (40.9%)	19 (55.9%)	0.122
Cerebrovascular disease <sup>b</sup>	19 (12.8%)	15 (13%)	4 (11.8%)	0.844
Peripheral arterial disease <sup>b</sup>	116 (77.9%)	85 (73.9%)	31 (91.2%)	0.033*
Renal insufficiency <sup>b</sup>	7 (4.7%)	7 (6.1%)	0 (0%)	0.141
Severity of ischemia				
Rutherford I	85 (57.0%)	64 (55.7%)	21 (61.8%)	
Rutherford IIa	50 (33.6%)	40 (34.8%)	10 (29.4%)	0.814
Rutherford IIb	14 (9.4%)	11 (9.6%)	3 (8.8%)	

<sup>a</sup>Independent-samples Mann–Whitney *U*-test.

<sup>b</sup>Pearson's chi-squared test for dichotomous variables.

\**P* < 0.05 (statistically significant).

short segment jump graft bypass) and/or endovascular manipulations (PTA, stenting) after effective thrombolysis was not considered as failure.

The failure criteria (failure group) comprised rapid progression of ischemia and major bleeding complications, both requiring thrombolysis termination and surgery. Inability to reopen native arteries/grfts, run-off vessels, in-hospital death, need for major amputation, and reocclusions within the 30-day follow-up period were also considered as failure. The incidence of major bleeding (intracerebral hemorrhage, intestinal bleeding, or puncture-site bleeding requiring surgical intervention) was assessed to evaluate the safety of thrombolysis.

### Data Analysis

Statistical analyses were carried out using the SPSS statistical software (SPSS, Inc., Chicago, IL, USA). Dichotomous variables were analyzed with the chi-squared test. Due to the skewed distribution of data, continuous variables were analyzed using the Mann–Whitney *U*-test, and thus the data are presented as median and range. To assess the association of various risk factors with thrombolysis outcome, both univariate and backward stepwise logistic regression analyses

were used. The latter was performed to evaluate the independent role of various factors in thrombolysis outcome. For stepwise criteria, a *P* value of 0.05 was specified for entry and 0.10 for removal. *P* < 0.05 was considered statistically significant. *P* < 0.06 was considered nearly statistically significant.

### RESULTS

Of the 149 patients who underwent thrombolysis during the study period, there were 79 men (53%) and 70 women (47%), with a median age of 70 (range 32–93) years. Table I shows the distribution of comorbidities and other variables between the groups. Hypercholesterolemia, previous bypass grafting, and peripheral arterial disease were significantly more frequent among those with thrombolysis failure.

Thrombolysis was successful in 115 (77%) of 149 cases. Thrombolytic therapy was sufficient as a monotherapy in 36 cases (24% of 149 cases), but in 60 cases (40%) an additional endovascular procedure was required to achieve adequate distal perfusion. Additional minor surgical operations after effective thrombolysis were done in 16 cases

**Table II.** Reasons for failure

Reasons	Frequency [N (%)]
Rapid progression of ischemic changes during the thrombolytic therapy requiring conversion to open surgery	4 (2.7%)
No lysis effect within 2 days of treatment	10 (6.7%)
Complete or partial lysis with inability to reopen run-off vessels	10 (6.7%)
Reocclusion within 1 month of thrombolysis	5 (3.4%)
Major amputation (above the metatarsal level) within 1 month of thrombolysis as a result of inability to obtain adequate distal perfusion	13 (8.7%)
Termination of thrombolysis due to major bleeding	2 (1.3%)
In-hospital death	4 (2.7%)

(10.7%) and a combination of thrombolysis, endovascular, and open minor surgery in 3 cases (2%). The minor amputation rate after successful thrombolysis was 0.7% ( $N = 1$ ).

The reasons for thrombolysis failure are summarized in Table II. In the failure group ( $N = 34$ , 23%) major reconstructive surgery was required in half of the group's cases ( $N = 17$ , 11.4%). All the amputations in the failure group were major ( $N = 13$ ), demonstrating an 8.7% rate within the whole patient group treated with thrombolysis. Four in-hospital deaths occurred in the course of treatment with alteplase (2.7% of 149 cases). Two patients died from septicemia, 1 from acute myocardial infarction and 1 from complications after massive thromboembolism. There were 2 major bleeding complications (puncture site), both managed surgically (1.3% of 149 cases).

In the univariate analysis, hypercholesterolemia, previous bypass grafting, and peripheral arterial disease were found to be significant predictors of thrombolysis failure. Prolonged duration of symptoms showed a nearly significant association (Table IIIa). In the backward stepwise logistic regression analysis including all the factors (Table IIIb), the parameters that independently impacted the success of thrombolysis leading to failure were hypercholesterolemia (odds ratio [OR] 0.16, 95% confidence interval [CI] 0.06–0.42,  $P < 0.0001$ ), previous bypass grafting (OR 0.18, 95% CI 0.06–0.53,  $P = 0.002$ ), and prolonged duration of symptoms (OR 0.95, 95% CI 0.91–0.99,  $P = 0.009$ , per day).

## DISCUSSION

In this study we found thrombolysis to be an effective treatment modality for patients with category I–IIb acute limb ischemia. The achieved success rate of 77% is an improvement compared with our institution's previously reported data (59.2%),<sup>13</sup> and is in accordance with results from other investigators.<sup>1–3,7</sup> Our study has also demonstrated that hypercholesterolemia, previous bypass grafting, and prolonged duration of symptoms can predict lower limb intra-arterial thrombolysis failure. Furthermore, our thrombolysis protocol seems to be safe as the major bleeding complications rate was only 1.3%.

The role of hypercholesterolemia in the outcome of thrombolytic therapy for acute lower limb ischemia has not been thoroughly studied. To our knowledge, only 3 research groups have assessed this subject previously, and they came to the conclusion that hypercholesterolemia plays no significant role in the sequelae of intra-arterial thrombolysis, thus contradicting our results.<sup>1,3,11</sup> It must be emphasized, however, that the aforementioned studies differ in regard to study end points, cohort size, patients' demographic data, and statistical analysis used, which makes it difficult to compare them with our study. Furthermore, as information concerning the presence of hypercholesterolemia was not available for all participants in the study by Plate and colleagues, any definitive conclusions on its role would be unjustified.<sup>1</sup> Nevertheless, the current data on vascular pathophysiology support the idea of hypercholesterolemia's impact on thrombolysis,<sup>14,15</sup> thus making the results of our study explainable.

Another major factor determining thrombolysis success is previous bypass grafting of the ipsilateral limb. Although occluded bypass grafts can often be successfully reopened with thrombolysis, reocclusion and long-term patency rates are poor.<sup>1,9,16,17</sup> Our findings support these earlier results. Worsened outflow as a result of PAD progression may partly explain this phenomenon. It has also been proposed that endothelium alterations in the bypass veins and the development of a thin prothrombotic layer in synthetic grafts could result in thrombolysis failure.<sup>18</sup>

A delay in treatment initiation is also thought to have a negative impact on the results of thrombolytic therapy.<sup>8,19,20</sup> The results of the STILE trial suggest that thrombolytic therapy may be offered to patients with acute symptoms lasting for no longer than 2 weeks. In our cohort there was no significant difference in the median duration of

**Table III.** Binary logistic regression analysis of the impact of risk factors on success of thrombolysis

Variables	OR	CI	P
(a) Univariate analysis			
Age	1.01	0.98–1.05	0.452
Gender	1.86	0.86–4.04	0.118
Hypertension	0.65	0.23–1.87	0.428
Atrial fibrillation	0.70	0.32–1.51	0.359
Heart failure	1.22	0.42–3.54	0.713
Hypercholesterolemia	0.16	0.06–0.39	<0.0001*
Diabetes	0.60	0.24–1.54	0.291
Smoking	0.50	0.22–1.12	0.092
Previous bypass grafting	0.29	0.12–0.67	0.004*
Aneurysm proximally	1.82	0.21–15.63	0.587
Previous vascular intervention within 1 month	0.64	0.18–2.21	0.478
Duration of symptoms	0.97	0.93–1.00	0.052
Severity of ischemia	1.18	0.65–2.13	0.596
Coronary disease	0.55	0.25–1.18	0.124
Cerebrovascular disease	1.13	0.35–3.65	0.844
Peripheral arterial disease	0.27	0.08–0.96	0.043*
Renal insufficiency	NA	NA	0.999
(b) Backward stepwise regression analysis			
Hypercholesterolemia	0.16	0.06–0.42	<0.0001*
Previous bypass grafting	0.18	0.06–0.53	0.002*
Duration of symptoms	0.95	0.91–0.99	0.009*

NA, not available.

\* $P < 0.05$  (statistically significant).

symptoms between the success and the failure groups (Table I). The backward stepwise regression analysis, however, showed that the duration of symptoms is an independent risk factor for thrombolysis failure and that the risk of failure increases by 5% per day. This finding may be weakened by the fact that symptom duration is a subjective measure as patients experience pain and sensory loss quite differently. This applies particularly to those with a development of sensory changes due to diabetic neuropathy.

There are several limitations to our study; the main drawback is its retrospective design, involving possible data issues associated with the use of hospital discharge histories and patient case records. Consequently, miscoding and a lack of clinical information may cause uncertainty in the results. However, the multiple admissions of the subjects to our hospital due to comorbidities prior to the initiation of the current study made data collection easier and, we believe, more accurate. Furthermore, possible data errors and miscoding would have been similar for all patients enrolled. Another potential issue is the selection bias, as patients were selected for thrombolysis according to the surgeon's clinical assessment with no strict inclusion or exclusion criteria. The selection of treatment options also

depended on the availability of the interventional radiologic service, which is not provided on a 24-hr basis at our hospital.

In conclusion, catheter-directed intra-arterial thrombolysis seems to be safe and effective in acute or subacute lower limb ischemia. Non-hypercholesterolemic patients with a short duration of ischemic symptoms presenting with thrombotic events of native arteries can benefit the most from thrombolytic treatment. However, when thrombolysis fails, the prognosis is poor as almost 40% (within the failure group) of patients treated require major amputations within 1 month. Our study has produced novel information about the possible role of hypercholesterolemia in the process of thrombolysis failure. This finding, however, requires more research before definitive conclusions can be drawn.

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# PUBLICATION

## II

**Bacterial signatures in thrombus aspirates of patients with lower limb arterial and venous thrombosis**

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# Bacterial signatures in thrombus aspirates of patients with lower limb arterial and venous thrombosis



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## ABSTRACT

**Objective:** Increasing data supports the role of bacterial inflammation in adverse events of cardiovascular and cerebrovascular diseases. In our previous research, DNA of bacterial species found in coronary artery thrombus aspirates and ruptured cerebral aneurysms were mostly of endodontic and periodontal origin, where *Streptococcus mitis* group DNA was the most common. We hypothesized that the genomes of *S mitis* group could be identified in thrombus aspirates of patients with lower limb arterial and deep venous thrombosis.

**Methods:** Thrombus aspirates and control blood samples taken from 42 patients with acute or acute-on-chronic lower limb ischemia (Rutherford I-IIb) owing to arterial or graft thrombosis (n = 31) or lower limb deep venous thrombosis (n = 11) were examined using a quantitative real-time polymerase chain reaction to detect all possible bacterial DNA and DNA of *S mitis* group in particular. The samples were considered positive, if the amount of bacterial DNA in the thrombus aspirates was 2-fold or greater in comparison with control blood samples.

**Results:** In the positive samples the mean difference for the total bacterial DNA was 12.1-fold (median, 7.1), whereas the differences for *S mitis* group DNA were a mean of 29.1 and a median of 5.2-fold. Of the arterial thrombus aspirates, 57.9% were positive for bacterial DNA, whereas bacterial genomes were found in 75% of bypass graft thrombosis with 77.8% of the prosthetic grafts being positive. Of the deep vein thrombus aspirates, 45.5% contained bacterial genomes. Most (80%) of bacterial DNA-positive cases contained DNA from the *S mitis* group. Previous arterial interventions were significantly associated with the occurrence of *S mitis* group DNA ( $P = .049$ , Fisher's exact test).

**Conclusions:** This is the first study to report the presence of bacterial DNA, predominantly of *S mitis* group origin, in the thrombus aspirates of surgical patients with lower limb arterial and deep venous thrombosis, suggesting their possible role in the pathogenesis of thrombotic events. Additional studies will, however, be needed to reach a final conclusion. (J Vasc Surg 2018;67:1902-7.)

**Clinical Relevance:** This is the first study to report the presence of bacterial DNA, predominantly of *Streptococcus mitis* group origin, in the thrombus aspirates of patients with lower limb arterial and deep venous thrombosis. Interestingly, the occurrence of *S mitis* group DNA seems to be significantly associated with previous vascular manipulations. It remains to be established whether these findings play a role in the actual thrombosis.

Bacterial inflammation has long been suggested to contribute to inflammation of atherosclerotic plaques, either directly or by indirect mechanisms, where inflammation at nonvascular sites can contribute to the progression of the lesions. Proposed bacteria include respiratory pathogens and periodontal bacteria as well as bacteria from the gastrointestinal tract.<sup>1</sup> One group of common oral bacterial species, namely, *Streptococcus mitis* group, has been shown to have an exceptional ability to adhere to an endothelial surface and bind

platelets as well.<sup>2,3</sup> Previous studies<sup>4-6</sup> showed that *S mitis* group DNA was predominant in thrombus aspirates from coronary arteries and cerebral arterial wall samples in patients with acute coronary events and cerebral aneurysms respectively. The *S mitis* group comprises 13 species.<sup>7</sup> Most of these bacteria can be found in the oral cavity of healthy individuals.

Based on these previous studies, it is likely that oral pathogens may play a certain role in the pathogenesis of cardiovascular diseases.<sup>4-6</sup> To the best of our

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knowledge, there are no studies depicting the role of dental or periodontal bacteria in the thromboembolic events of lower limbs and whether they are present in venous thrombosis. Thus, the target bacteria for our study were *S mitis* group because their DNA could be found in almost 80%<sup>4</sup> of the thrombus aspirates and in the majority of the arterial wall samples<sup>5,6</sup> of the patients with cardiovascular and cerebrovascular diseases, respectively. We hypothesize that the signs of these oral pathogens can equally be found in the thrombus aspirates of peripheral vessels of the lower limbs.

## METHODS

### Patients

This prospective study analyzed data from consecutive patients (N = 62) referred to Tampere University Hospital, Tampere, Finland, for acute or acute-on-chronic lower limb arterial, arterial bypass graft, or deep venous thrombosis from September 2014 to October 2016.

**Inclusion criteria.** Patients with symptoms or signs of acute or acute-on-chronic lower limb ischemia (Rutherford class I-IIb)<sup>8</sup> presenting with angiographic evidence of native artery or bypass graft thrombosis (n = 31) and patients presenting with deep venous thrombosis of the iliofemoral segment with symptoms' duration no longer than 2 weeks (n = 11).

**Exclusion criteria.** Patients with progressive sensorimotor changes or contraindications for thrombolysis (n = 20). Those patients were treated surgically and excluded from further analysis. Based on our previous experience, thrombus aspirates from those patients were not obtained owing to increased sample contamination risk.<sup>9,10</sup> The patients included in the study were treated with thrombolysis, and the actual study group thus comprised 42 cases (Table). The study was approved by the ethics committee of the hospital, and all the patients included gave informed consent.

### Specimen processing

Aspiration of thrombus samples was performed aseptically in an angiography laboratory by an interventional radiologist. The antiseptic routinely used for skin disinfection was a denatured ethanol solution (A12T Dilutus 80%; Berner, Helsinki, Finland) being effective against both aerobic and anaerobic pathogens.<sup>11</sup> Patients received no antibiotic therapy at admission to the hospital or later during the procedure. The procedure was carried out within 3 hours after admission to the hospital in cases of acute arterial ischemia and within 12 hours in venous cases, if no significant venous ischemia or life-threatening conditions were present. An introducer sheath was placed, and the thrombus aspirates were obtained using 6-F angio catheters from the proximal parts of the arteries or grafts and usually distally in the cases of deep venous thrombosis before initiation of

## ARTICLE HIGHLIGHTS

- **Type of Research:** Prospective cohort study
- **Take Home Message:** Aspirates from acute arterial, bypass graft or deep vein thrombus contained bacterial DNA in 25 of 39 patients (64.1%), most frequently (77.8%) from acute thrombus formed in prosthetic bypass grafts. The majority 80% of bacterial DNA-positive cases contained DNA from the *Streptococcus mitis* group
- **Recommendation:** These data support the possible role of bacterial inflammation, caused predominantly by *S mitis*, in thrombotic events.

thrombolysis and placed into Eppendorf tubes. Control blood samples were taken through the introducer sheaths before the thrombus aspiration took place and stored in similar tubes. The specimens were frozen at  $-80^{\circ}\text{C}$  after collection. DNA from the samples was extracted using a commercial QIAmp DNA Mini Kit (Qiagen Ltd, Calif) according to the instructions provided. Blood and whole collected thrombus aspirates were then analyzed using the real-time quantitative polymerase chain reaction (qPCR). The risk for contamination was reduced to minimal as the specimen handling was performed aseptically throughout the whole process.

### Real-time qPCR

The presence of bacterial DNA was identified using qPCR with ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, Calif) as previously described<sup>12</sup> with Maxima Probe/ROX qPCR Master-Mix (Thermo Fischer Scientific, Waltham, Mass). Arterial thrombus aspirates were compared with arterial control blood samples, as opposed to venous to venous to reduce any potential bias caused by sampling from different sites and, subsequently, bias resulting from different conditions like flow dynamics and pressure. The presence of bacterial DNA in thrombus and in control blood samples were determined by using published primers and a probe for *Streptococcus* spp., mainly *S mitis* group,<sup>12</sup> and universal bacterial primers and a probe<sup>13</sup> using human housekeeping gene, RNaseP (Applied Biosystems), as a reference gene. Each measurement was performed as duplicates or quadruples in uncertain cases. The relative amounts of bacterial DNA in samples were calculated by the comparative threshold cycle (Ct) method ( $\Delta\Delta\text{Ct}$ ,  $\Delta\text{Ct}_{\text{sample}} - \Delta\text{Ct}_{\text{control}}$ ),<sup>14</sup> where the sample was a thrombus aspirate and control was a blood sample from the same patient. First, the differences of the Ct values ( $\Delta\text{Ct}$ ) between candidate bacteria and reference gene measurement (Ct from candidate bacteria - Ct from RNaseP) for each sample were calculated; then the comparative Ct ( $\Delta\Delta\text{Ct}$ ) ( $\Delta\text{Ct}$  from thrombus -  $\Delta\text{Ct}$  from one patient's own arterial blood)

**Table.** Patient characteristics and association of bacterial DNA occurrence with various parameters

Characteristics	All patients (N = 42)	Positive total bacterial DNA (n = 25)	Positive <i>S mitis</i> group DNA (n = 23)
Median age (range), years	70 (38-92)	69 (38-92)	67 (38-92) <sup>a</sup>
Male gender	24 (57.1)	13 (52.0)	14 (60.9) <sup>b,c</sup>
Diabetes	12 (28.6)	8 (32.0)	6 (26.1) <sup>b,c</sup>
Dyslipidemia	19 (45.2)	12 (48.0)	11 (47.8) <sup>b,c</sup>
Arterial hypertension	31 (73.8)	21 (84.0)	17 (73.9) <sup>b,c</sup>
Coronary heart disease	6 (14.3)	4 (16.0)	4 (17.4) <sup>b,c</sup>
Cerebrovascular disease	4 (9.5)	3 (12.0)	3 (13.0) <sup>b,c</sup>
Pulmonary disease	10 (23.8)	7 (28.0)	7 (30.4) <sup>b,c</sup>
Renal insufficiency	4 (9.5)	3 (12.0)	3 (13.0) <sup>b,c</sup>
Previous arterial procedure	19 (45.2)	14 (56.0)	14 (60.9) <sup>b,c,d</sup>
Arrhythmias	10 (23.8)	4 (16.0)	4 (17.4) <sup>b,c</sup>
Heart failure	4 (9.5)	2 (8.0)	2 (8.7) <sup>b,c</sup>
Smoking within 5 years	19 (45.2)	14 (56.0)	12 (52.2) <sup>b,c</sup>
Type of thrombus aspirate			
Vein	11 (26.2)	5 (20.0)	5 (21.7) <sup>b,c</sup>
Artery	19 (45.2)	11 (44.0)	10 (43.5) <sup>b,c</sup>
Arterial bypass graft	12 (28.6)	9 (36.0)	8 (34.8) <sup>b,c</sup>
Autologous vein	3 (7.1)	2 (8.0)	2 (8.7) <sup>b,c</sup>
Prosthesis	9 (21.4)	7 (28.0)	6 (26.1) <sup>b,c</sup>
Median symptom duration (range), days	7 (1-30)	7 (1-30)	7 (1-30) <sup>a</sup>

Data are presented as number (%) unless otherwise indicated.  
<sup>a</sup>Mann-Whitney *U* test.  
<sup>b</sup>Fisher's exact test.  
<sup>c</sup>Pearson  $\chi^2$ .  
<sup>d</sup> $P < .05$ .

was calculated. The samples were marked bacterial positive, if  $2^{-\Delta\Delta Ct} \geq 2$ .

### Comorbid conditions

Data from patients and files were collected systematically upon admission to the hospital by one examiner (D.V.). The following risk factors from their case records were included in the analysis: age, sex, diabetes mellitus, dyslipidemia, hypertension, coronary heart disease, cerebrovascular disease, pulmonary diseases, renal insufficiency, history of previous arterial interventions (>1 month old), heart rhythm disorders, heart failure, smoking within 5 years, and duration of symptoms. The diagnoses for each of the diseases were cross-checked with the patient and/or considered positive if they had been established previously at the hospital or mentioned in the referral, or if the patient was on an appropriate medication.

### Data analysis

Statistical analyses were carried out using the SPSS Statistics (SPSS, Chicago, Ill) software package. Fisher's exact test and Pearson  $\chi^2$  tests were used for nominal parameters. Independent samples Mann-Whitney *U* test was used to analyze the association of continuous

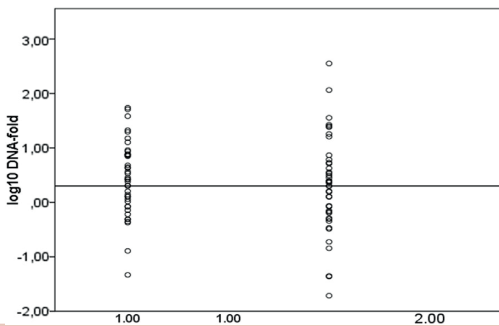
parameters (age, symptoms duration) with the presence of bacterial DNA. Statistical significance was set at  $P \leq .05$ .

### RESULTS

Altogether, 42 thrombus and control blood samples accordingly were collected and analyzed. There were 24 specimens from men (57.1%). Of the 42 thrombus aspirates obtained, three were excluded from further statistical analysis for associations of total bacterial DNA with various parameters owing to technical failure in sample processing. In two of those samples, the evaluation of *S mitis* group DNA was impossible for the same reason.

The samples were considered positive, if the amount of bacterial DNA in the thrombus aspirates was twofold or greater<sup>15</sup> in comparison with control blood samples. Thus, for the total bacterial DNA the mean fold difference was 12.1 (median, 7.1), whereas the differences for *S mitis* group DNA were a mean of 29.1 and a median of 5.2-fold (Fig).

Distributions of comorbidities and other variables are presented in the Table. The total prevalence of any bacterial DNA in successfully processed samples of thrombus aspirates was 64.1% (n = 25; bacteria not specified and include all possible microorganisms). Of the 19 arterial thrombus aspirates, 11 (57.9 %) were positive for bacterial



**Fig.** Relative amounts of bacterial DNA in patients' thrombus aspirates. N-fold difference (log<sub>10</sub>) compared with the subject's own peripheral blood. The horizontal line shows the bacterial positivity level (2-fold difference). *Column 1*: Total Bacterial DNA. *Column 2*: *S mitis* group DNA.

DNA. Bacterial genomes were found in 9 of 12 bypass graft thrombosis specimens (75%) with 7 of 9 prosthetic graft thrombi (77.8%) being positive. Of the deep vein thrombus aspirates, 5 of 11 (45.5%) contained bacterial genomes. The majority, 20 of 25 bacterial DNA positive cases (80%), contained DNA from *S mitis* group. There were no previous venous interventions in the group examined; however, previous arterial intervention was found to be associated with the occurrence of *S mitis* group, whereas the associations of bacterial DNA presence with other parameters were not significant (Table).

## DISCUSSION

To the extent of our knowledge, this is the first study to demonstrate the presence of bacterial DNA in thrombus aspirates of patients with symptomatic lower limb arterial and acute deep venous thrombosis. We also demonstrated that 80% of bacterial DNA was from the *S mitis* group. Our findings are in accordance with the results of bacterial DNA analysis of thrombus aspirates from the coronary arteries of patients with myocardial infarction and specimens taken from the intracranial aneurysms.<sup>4,6</sup> This study has also shown that previous arterial manipulations were significantly associated with the presence of *S mitis* group DNA.

There has been growing evidence in favor of the role of oral bacteria in the pathogenesis of atherosclerosis.<sup>16</sup> Furthermore, *S mitis* group, as a representative of oral bacterial species, seems to have an exceptional ability to bind platelets causing their aggregation.<sup>2,3,17,18</sup> At the same time, bacteria from this group can adhere to an endothelial surface creating inflammatory and thrombogenic biofilms that allow other bacteria to attach as well,<sup>19</sup> which, in turn, can potentially escalate the effect of platelet aggregation. In contrast, there is evidence that the platelets themselves play an important role in the response to infection by binding to the bacterial surface, aggregating and secreting antibacterial

peptides and cytokines to recruit immune cells to eliminate (phagocytize) the bacterial agents.<sup>18,20,21</sup> Oral bacteria can access the bloodstream through the endothelial lesions in the oral cavity and as a result of dental infections. Thus, a significant association between the presence of a periapical dental abscess and *S mitis* group DNA in the coronary thrombus aspirates in patients with acute myocardial infarction was established.<sup>4</sup> Bacteremia may, however, occur not only in periodontitis patients, but in individuals with no significant periodontal disease as well.<sup>22</sup> It was demonstrated that dental procedures, tooth brushing, and even gum chewing may produce bacteremias of orally derived microorganisms.<sup>23,24</sup> Odontogenic bacteria have, therefore, been found on heart valves, endocardium, or atherosclerotic plaques.<sup>18</sup>

These mechanisms seem logical in the situations where thrombotic events occur in the arteries, where we deal with the overt disruption of the intima owing to atherosclerotic plaques or previous arterial procedures. Thus, a prosthetic bypass graft, being a foreign surface, is a preferred target for bacterial adherence,<sup>25</sup> despite the absence of a natural endothelial layer, which is substituted by neointima. Neoendothelialization of polytetrafluoroethylene grafts includes phases of platelet aggregation and fibrin network formation.<sup>26</sup> Bacteria, in turn, are known to have an ability to adhere to platelets and fibrin.<sup>27</sup> Furthermore, in this work the thrombus samples were obtained exactly from the proximal parts of the grafts near the anastomosis area, which usually contains natural intima that could provide bacteria with a suitable adherence surface.

The role of bacterial presence in thrombus formation is not understood completely. Frequent bacteremia events may result in an accumulation of pathogens in atherosclerotic plaques with a development of a chronic low-grade inflammation.<sup>4</sup> Rupture of a plaque inhabited by microorganisms with a platelet binding potential could, in turn, lead to a clot formation.

Deep venous thrombosis may have similar characteristics, even though the overt mechanical endothelial disruption is usually absent. According to the postulates of Virchow's triad,<sup>28</sup> slow velocity in the venous flow may be one of the reasons of thrombosis, which is described in a mathematical model performed by Elizondo et al.<sup>29</sup> Deep venous thrombosis of the lower limb usually occurs in the area of a valve, where the flow separation takes place and results in recirculation and interaction of blood cells.<sup>30</sup> It is likely that the presence of bacteria in a slow-flow, semi-isolated venous segment would probably induce platelet activation with their aggregation, thus, possibly, playing a role in subsequent thrombosis. Another route for bacteria to enter atherosclerotic plaques or larger vein walls during a bacteremia of any origin could be vasa vasorum, which surround vascular structures from the adventitial side.

Possible limitations of this study include the fact that it reports the presence of bacterial DNA in the examined samples, but it does not reveal the presence of living microorganisms and/or their impact on blood clotting. Culturing and staining, often used to detect the bacterial species, in our study, unfortunately, had limited potential; thus, qPCR was used instead. Culturing has a very low sensitivity, from  $10^3$  to  $10^4$  of vital bacteria are required to form a culture.<sup>31</sup> Cultivating anaerobic species is even more complicated, and the microorganisms related to the periodontal disease, in turn, are predominantly anaerobic.<sup>31</sup> Previously performed studies<sup>32,33</sup> have demonstrated PCR methods in detecting bacterial signs to be more accurate, time consuming, and cost effective in comparison with cultures. Furthermore, based on our previous experience with staining and electron microscopy, revealing whole bacterial structures in the clot aspirates was not always definite,<sup>4</sup> because bacteria or bacterial biofilms, if present, could mainly reside on the endothelial or plaque surfaces and, thus, the distinctly increased amounts of bacterial DNA in the thrombus are, possibly, those "on-site" phagocytized bacterial components from the biofilms.

It is clear that the presence of bacterial DNA in thrombus aspirates can also occur if the binding of platelets and bacteria, alternatively, takes place somewhere else within the blood stream before a thrombus adheres to an endothelial surface. Besides adhesion, aggregation of platelets and formation of a clot on an endothelial surface may attract inflammatory cells with elsewhere phagocytized bacterial DNA fragments. However, a multi-fold difference in the amounts of bacterial DNA found in thrombus aspirates suggests their possible role in the pathogenesis of thrombosis. Nonetheless, how significant this role is remains to be studied by additional methods.

Another weakness of this study is its relatively small number of cases, which prevents us from performing further subgroup analysis.

In conclusion, our study is among the first to identify the DNA of the microorganisms of the oral cavity in the blood clots of the lower limb vessels. We suppose that species like *S mitis* group could be associated with the incidence of arterial and, possibly, lower limb deep venous thrombosis by means of initiation of inflammation in the vessel wall leading to plaque or endothelial alterations that may eventually result in thrombotic occlusions. The role of bacteria in venous thrombosis is yet to be studied but, apparently, inflammation might play a key role. The results of the study are interesting but, nonetheless, should be interpreted with caution as additional studies will be needed to reach the final conclusion.

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## AUTHOR CONTRIBUTIONS

Conception and design: DV, ST, MM, JK, TP, JS, VS, TL, PK, NO

Analysis and interpretation: DV, ST, MM, TP, TL, PK, NO

Data collection: DV

Writing the article: DV, ST, TP, PK, NO

Critical revision of the article: DV, ST, MM, JK, TP, JS, VS, TL, PK, NO

Final approval of the article: DV, ST, MM, JK, TP, JS, VS, TL, PK, NO

Statistical analysis: DV, NO

Obtained funding: DV, TL, PK

Overall responsibility: DV

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# PUBLICATION III

**Survival of patients and treatment-related outcome after intra-arterial  
thrombolysis for acute lower limb ischemia**

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# Survival of Patients and Treatment-Related Outcome After Intra-Arterial Thrombolysis for Acute Lower Limb Ischemia

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**Background:** The aim of this study is to evaluate the long-term survival and treatment-related outcome in patients treated with intra-arterial thrombolysis for acute lower limb ischemia.

**Methods:** The study was based on a prospective vascular database with retrospectively obtained supplementary information from the patients' files. Additionally, data on the patients' date and cause of death were obtained from Statistics Finland. A total of 155 patients with symptoms or signs of category I–IIa acute lower limb ischemia and angiographic evidence of native artery or bypass graft thromboembolic events were treated with intra-arterial catheter-directed thrombolysis (CDT). Patients with severe ischemic stages at admission or those with contraindications for thrombolysis ( $n = 185$ ) were treated with conventional surgical modalities and excluded from further analysis.

**Results:** The mean age of the patients at admission was 73 years (95% confidence interval 70.1–74.6). For descriptive purposes, age quartiles were used ( $\leq 64$ , 65–74, 75–82.5,  $\geq 83$ ). The mean follow-up time was 126.3 months. The primary patency rates of native arteries/bypass grafts were 59.8%/31.7%, 35.4%/17.1%, and 18.7%/15.2% at 1, 5, and 10 years, respectively ( $P = 0.01$ ). Correspondingly, the respective secondary patency rates were 65.2%/55.6%, 46.7%/39.8%, and 22.8%/30.5% ( $P = 0.88$ ). A total of 190 additional procedures on 122 patients were required to preserve the patency after hospital discharge. At 1 year the cumulative survival was 78%, at 5 years 56%, and at 10 years 29%. The most common cause of death was cardiovascular (68.5%), predominantly presented by an acute coronary syndrome, while 9.6% died of cancer, 6.8% of pulmonary diseases, 8.2% of cerebrovascular causes, and 19.2% owing to trauma and other reasons. Atrial fibrillation (hazards ratio [HR] 2.31) and age over 83 years (HR 5.23 per age category) were significantly and independently associated with poorer cumulative post-procedural survival. Bypass graft thrombosis was associated with an increase in major amputations after CDT (HR 14.77). However, the presence of synthetic bypass grafts had a protective influence on limb salvage (HR 0.086). A total of 39 (25.2%) major amputations were performed during the follow-up period. Age over 75 years was the only significant and independent factor to negatively impact on amputation-free survival (HR 2.01), which was 24% at 10 years.

**Conclusions:** The long-term patency after CDT is unfavorable, and additional procedures are needed to preserve adequate distal perfusion. Approximately 30% of the patients are alive at 10 years after the initial CDT. Increasing age and atrial fibrillation have a negative effect on the patients' survival.

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## INTRODUCTION

Intra-arterial thrombolysis for the treatment of lower limb thromboembolic events was introduced over 2 decades ago and has been routinely used ever since. The procedure has been shown to be safe and effective.<sup>1–6</sup> However, almost 40% of the treated patients develop irreversible ischemic changes, which require major amputations within the first month if the initial thrombolytic therapy fails.<sup>5</sup>

Some studies have analyzed the role of comorbidities as well as other factors on the short- and long-term outcome after thrombolysis<sup>3,4,7–9</sup> and conventional surgical treatment modalities.<sup>3,9,10</sup> Nonetheless, the majority of the works assessing endovascular treatment outcomes focus on amputation-free survival as their end point and present only short- or mid-term results. The studies differ in cohort sizes, end points, and the statistical methods used. It is notable that many analyses reveal advanced age as an independent factor associated with mortality.<sup>3,4,6,8,9</sup> The presence of other factors varies and is not consistent. In a large study by Earnshaw et al.,<sup>3</sup> factors that were shown to be associated with poor post-procedural survival included female sex, increasing age, native vessel occlusion, and occlusions of embolic origin. Other investigators, in turn, found renal insufficiency, ischemic heart disease, cerebrovascular disease, and the presence of a foot ulcer to be independently associated with death during the follow-up.<sup>7</sup> The role of atrial fibrillation (AF) in post-thrombolytic survival is undefined, and the data available on this issue are scarce.

Patients with acute limb ischemia often have multiple comorbidities that can affect the prognosis negatively despite successfully performed lytic therapy.<sup>3</sup> Furthermore, acute lower limb ischemia (ALLI) could indicate the start of a cascade leading to the end of life, perhaps suggesting a different approach or, sometimes, palliative care instead.<sup>11</sup>

To the extent of our knowledge, the data available concerning the long-term outcomes of CDT are scarce. Therefore, the aim of this study is to assess the long-term patency, the need for major amputations, survival, as well as factors possibly affecting the outcomes.

## MATERIALS AND METHODS

### Patients

A total of 340 patients were referred to Tampere University Hospital (TAUH), Tampere, Finland, for

ALLI between March 2003 and December 2014. Patients with symptoms or signs of category I–IIa ALLI<sup>12</sup> and angiographic evidence of bypass graft or native arterial thrombosis were treated with intra-arterial catheter-directed thrombolysis (CDT) with alteplase (Actilyse; Boehringer Ingelheim, Ingelheim, Germany) and included in the study group ( $n = 155$ ). This group comprised all the patients initially treated with CDT. Patients with contraindications for thrombolytic therapy, those in whom an endovascular procedure was unavailable for various reasons, as well as patients with severe stages of ischemia at admission ( $n = 185$ ) were treated using conventional surgical modalities. Owing to heterogeneity, these patients were excluded from further analysis.

### Data Collection

The study was based on the local vascular database registry where the patient-related information had been entered prospectively. The data were cross-checked with the case records and, if needed, the authors (D.V. and E.S.) obtained additional information from the patients' case records retrospectively. The diagnosis for each disease was considered positive if it had preexisted in the patient's hospital records, if it had been mentioned in the referral, or if the patient was on appropriate medication. The data on the patients' causes and dates of death were obtained from Statistics Finland.

The data on amputations performed after the treatment for ALLI were collected from the vascular database registry and the patients' electronic case records. Only major amputations above the foot level were analyzed.

The Ethics Committee of TAUH approved the study (ethical and other patient's data-related issues). Informed consent from the patients was not required.

### Study End Points

The primary end points were the primary, assisted primary, and secondary patencies, in addition to the major amputation rate as well as the cumulative and amputation-free survival rates together with the causes of death in patients initially treated with thrombolysis. The secondary objective was to evaluate the factors associated with a poor post-procedural outcome.

### Definition of Patency

The definition of patency was based on the guidelines of Rutherford et al.<sup>12</sup> Patency was assessed

using objective clinical examination methods (ultrasound and/or angiography or ankle-brachial index measurement along with a clinical examination). These data were documented in the patients' history and partly also in the vascular registry.

Additional endovascular or minor open surgical procedures were performed in a number of patients upon the completion of thrombolysis in order to obtain sufficient distal perfusion. The endovascular procedures included balloon dilatations and stentings. The minor surgical procedures comprised endarterectomies, open angioplasties, and/or short-segment jump bypasses. The need for such additional procedures within the same hospital stay after thrombus dissolution with CDT was still defined as primary patency.

### Statistical Analysis

Kaplan-Meier survival analysis was used to estimate the patency, survival, and amputation-free survival. Standard error of <10% was considered reliable. Cox regression proportional hazards analysis was used to study the effect of different factors on survival. The parameters were first tested in the univariable model, and significant parameters ( $P < 0.05$ ) were included in the multivariable analysis. The factors were tested for proportional hazards assumption with a log-minus-log plot, and it was met for all the factors included in the multivariable model. Age was evaluated as a continuous parameter. For descriptive purposes, the age parameter was divided into age quartiles ( $\leq 64$ , 65–74, 75–82.5,  $\geq 83$ ).

## RESULTS

Altogether, 155 consecutive patients (73 men, 47.1%) were included in the study. Of 155 CDTs performed, 80 patients (51.6%) required additional manipulations in order to obtain sufficient distal perfusion upon the completion of the procedure. Those modalities included 58 percutaneous balloon dilatations/stentings, 20 minor surgical procedures, and 2 hybrid procedures. Moreover, 2 fasciotomies were performed upon the completion of thrombolytic treatment. Major bleeding requiring an early termination of the CDT, a blood transfusion, and surgical hemostasis occurred in 4.2% of the cases. Additionally, 2 more patients developed an intracranial hemorrhage and died within days of the procedure (1.3%).

The patients' characteristics are presented in Table I. The mean follow-up was 126.3 months

(95% confidence interval [CI] 111.6–141.1). As expected, the most common cause of death was related to cardiovascular reasons (68.5%), predominantly presented by an acute coronary syndrome. Other reasons included cancer (9.6%), cerebrovascular events (8.2%), pulmonary diseases (6.8%), and trauma and other causes (19.2%).

### Patency

A total of 190 additional procedures on 122 patients were required to preserve the patency after hospital discharge within the 10-year period.

The long-term patency rates were generally unfavorable. The primary patency of native arteries was better than that of bypass grafts at 1, 5, and 10 years post-procedurally ( $P = 0.01$ ; Fig. 1). The assisted primary patency of native vessels was also superior to bypass grafts after the treatment with CDT ( $P = 0.03$ ; Fig. 2). The secondary patency did not differ significantly between the native arteries and bypass grafts ( $P = 0.88$ ; Fig. 3).

### Survival

The cumulative survival analysis of the patients treated with CDT is demonstrated in Figure 4. The analysis shows that 78% of patients were alive at 1 year. This number decreased to 56% at 5 years, whereas 29% of patients were alive at 10 years after the treatment with CDT.

**Univariable analysis.** Age over 83 years, heart failure, diabetes, coronary heart disease, AF, arterial hypertension, renal insufficiency, and a previous arterial procedure of the ipsilateral limb were significantly associated with mortality. The presence of a bypass graft was associated with longer survival.

**Multivariable Cox regression analysis.** Age over 83 years was significantly and independently associated with poor post-procedural survival (hazards ratio [HR] 5.23 per age category, 95% CI 2.28–12.01,  $P = 0.000$ ). Furthermore, AF was significantly and independently associated with poor post-thrombolytic survival (HR 2.31, 95% CI 1.40–3.82,  $P = 0.001$ ).

### Major Amputations

A total of 39 (25.2%) major amputations were performed during the follow-up period. Ten of these (25.6%) were performed within the first month from the procedure, when sufficient distal perfusion could not be achieved.

**Univariable analysis.** Factors that predicted major amputations in patients treated with CDT included

thrombosis of autologous vein bypasses and the presence of additional vascular procedures within 6 months after initial lytic therapy.

**Multivariable Cox regression analysis.** The occurrence of graft thrombosis was associated with an increased risk of major amputation after CDT (HR 14.77, 95% CI 1.86–117.52,  $P = 0.011$ ). Nonetheless, the presence of synthetic bypass grafts seemed to protect against amputations in patients treated with CDT (HR 0.086, 95% CI 0.011–0.699,  $P = 0.022$ ).

### Amputation-free Survival

The amputation-free survival was 66%, 47%, and 24% at 1, 5, and 10 years post-procedurally (Fig. 5).

**Univariable analysis.** Age over 75 years, female sex, heart failure, smoking, AF, diabetes, coronary heart disease, and renal insufficiency had an adverse effect on the survival of patients after CDT. In contrast, the presence of a synthetic bypass graft demonstrated a protective effect.

**Multivariable Cox regression analysis.** Age over 75 years turned out to be significant and independent as an adverse factor affecting the survival after CDT (HR 2.01 per age category, 95% CI 1.03–3.92,  $P = 0.042$ ). The influence increased with age, since the patients aged over 83 years had an HR of 3.84 per age category (95% CI 1.95–7.58,  $P = 0.000$ ).

## DISCUSSION

To the extent of our knowledge, this study is one of the few to focus on the long-term post-procedural survival and patency after thrombolysis for acute lower limb arterial thrombotic events.

The study revealed that the patients died predominantly owing to acute coronary events and cancer. Furthermore, the study demonstrated that age over 83 years, as well as AF, were significantly and independently associated with poor post-procedural survival. Beyond that, the presence of occluded bypass grafts predicted an increased risk of major amputation.

Elderly patients presenting with acute lower limb thromboembolic events have been shown to have poor survival in the short, medium, and long term.<sup>7–9,13,14</sup> In the recent work by Grip et al.,<sup>8</sup> increasing age was a significant factor associated with death within 5 years from thrombolysis (odds ratio 1.07/year). Our results are in line with this finding (HR 5.23 per age category), demonstrating poorer mid- and long-term post-lytic

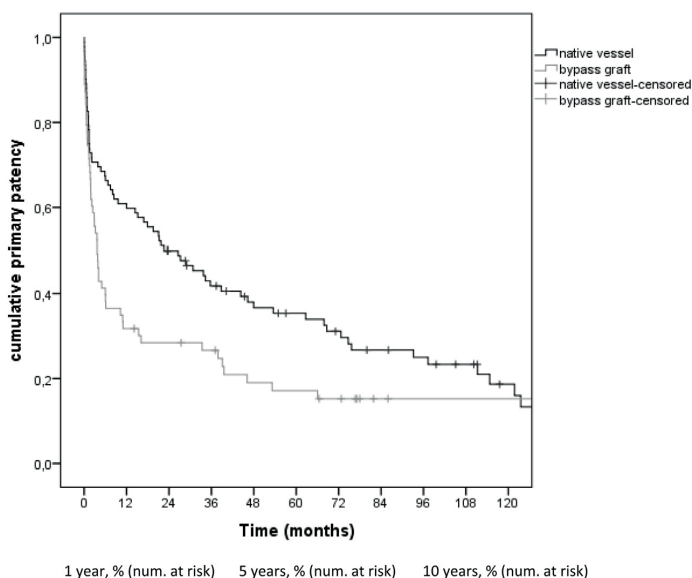
**Table I.** Patients' characteristics

Variables	Catheter-directed thrombolysis (n = 155)
Age (years), mean	72.7 (12.5)
Female sex, n (%)	82 (52.9)
Heart failure, n (%)	37 (23.9)
Smoking within 5 years	26 (16.8)
Atrial fibrillation, n (%)	70 (45.2)
Diabetes, n (%)	34 (21.9)
Dyslipidemia, n (%)	78 (50.3)
Arterial hypertension, n (%)	120 (77.4)
Coronary heart disease, n (%)	69 (44.5)
Cerebrovascular disease, n (%)	21 (13.5)
Pulmonary disease, n (%)	22 (14.2)
Renal insufficiency, n (%)	15 (9.7)
Previous bypass grafting, n (%)	63 (40.6)
Autologous vein graft	17 (11.0)
Synthetic graft	46 (29.7)
Occluded segment, n (%)	
Iliac	15 (9.7)
Femoral	11 (7.1)
Femoropopliteal	98 (63.2)
Tibial	31 (20.0)
Lytic therapy + additional procedure, n (%)	80 (51.6)
Secondary procedure within 6 months, n (%)	23 (14.8)
Severity of acute ischemia	
Rutherford I	57 (36.8)
Rutherford IIa	98 (63.2)
Duration of symptoms (days), mean	4.7 (5.5)
Duration of thrombolytic therapy (days), mean	1.5 (0.5)

Mean presented as values and standard deviation in brackets.

survival. This is true particularly for those over 83 years of age. Increasing age is, however, a natural, nonmodifiable factor. It is therefore understandable and logical that advanced age has been found to be associated with mortality after thrombolysis in most studies performed in the last 2 decades.<sup>2,3,5,7,8</sup>

The impact of AF on mortality in patients with ALLI treated with CDT is not well defined. Furthermore, the data available on this issue are scarce. AF has been found to be associated with death within 5 years from thrombolysis for ALLI.<sup>8</sup> Other recent studies, however, have not found it to be significant.<sup>6,7,14</sup> Nonetheless, AF has been revealed to correlate with an increased risk of cardiovascular disease.<sup>15</sup> In a large study by Benjamin et al.,<sup>16</sup> AF was associated with a 1.5- to 1.9-fold mortality risk after adjustment for the preexisting cardiovascular



	1 year, % (num. at risk)	5 years, % (num. at risk)	10 years, % (num. at risk)
Native artery, N=92	59.8 (55)	35.4 (25)	18.7 (7)
Bypass graft, N=63	31.7 (20)	17.1 (9)	15.2 (1)

**Fig. 1.** Primary patency after CDT for native arterial (black curve) or bypass graft (gray curve) thrombolysis. Kaplan-Meier survival analysis ( $P = 0.01$ ).

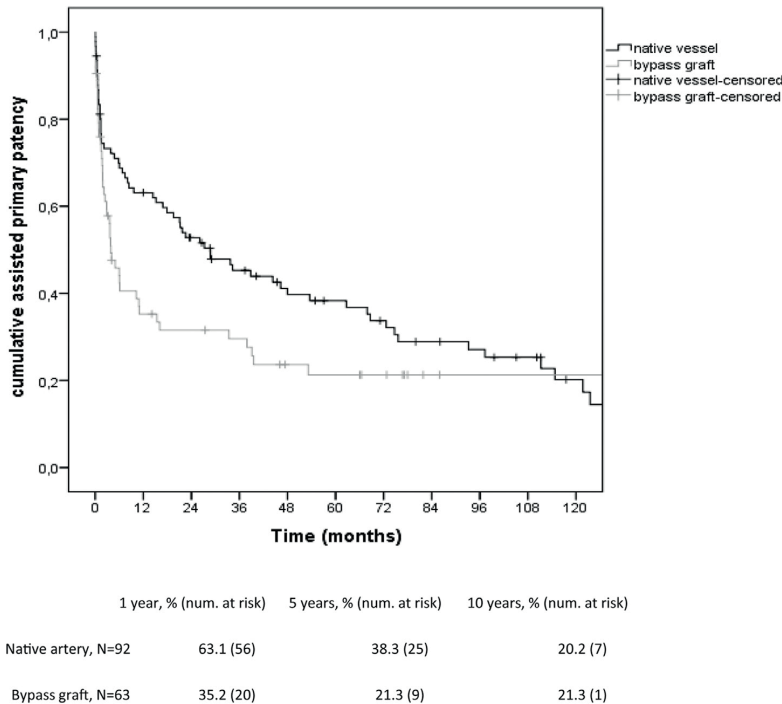
conditions with which AF was related. AF is known to have common risk factors and often coexists with congestive heart failure, whereas the presence of both conditions identifies patients at an increased mortality risk.<sup>17</sup> In another large study from Sweden that included over 200,000 patients with incidental AF, AF was an independent risk factor of all-cause mortality,<sup>18</sup> which supports our finding. It is noteworthy that the majority of our patients with AF received warfarin and the diagnosis remained present throughout the surveillance period. It remains debatable whether those patients could have benefited more if they had been prescribed a different anti-thrombotic therapy. Interestingly, patients with stable atherosclerotic disease receiving rivaroxaban in addition to aspirin have been recently reported<sup>19</sup> to have a 24% lower incidence of cardiovascular death, stroke, or myocardial infarction, but, expectedly, a higher risk of bleeding than those taking aspirin or rivaroxaban alone.

Coronary heart disease, cerebrovascular disease, diabetes, and renal insufficiency, despite having previously been described as significant factors affecting survival<sup>7,8</sup> in ALLI patients, did not turn out to be significant in this study. This could be

explained by differences in cohort sizes and the considerably longer surveillance in our group. Furthermore, the results may be affected by the fact that we do not have complete information on the management of risk factors among these patients nor on the patients' commitment to taking the prescribed medication.

Some authors have shown female sex<sup>3</sup> to have a negative impact on survival after thrombolysis. This finding is, however, contradicted by other investigators<sup>6,7,12</sup> and the results of our study as well. In general, women tend to live longer even with the same risk factor profiles as men, so we are inclined to believe that long-term survival after thrombolysis for ALLI could probably be better among women. To resolve this question, larger prospective cohorts would be needed.

Severe stages of ischemia have previously been shown to have an adverse effect on survival.<sup>8,9</sup> In our institution, we tend to manage patients with severe stages of ischemia surgically.<sup>12</sup> Consequently, these patients were excluded from this study, and we therefore lack reliable data on the outcome of these patient groups after CDT. Nonetheless, the less severe stages analyzed in our cohort did not significantly affect the survival of the patients,



**Fig. 2.** Assisted primary patency after CDT for native arterial (black curve) or bypass graft (gray curve) thrombosis. Kaplan-Meier survival analysis ( $P = 0.03$ ).

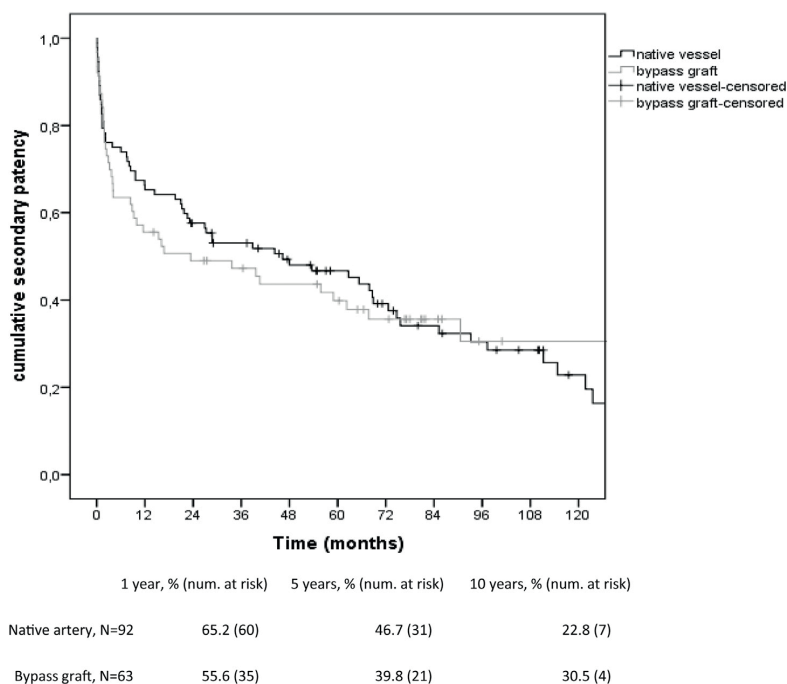
which is in line with the previously published data.<sup>5,8,9,13</sup>

According to our results, the presence of occluded bypass grafts seemed to increase the risk for major amputations after CDT. We suppose that this is true particularly for autologous vein grafts, since it has been suggested that they develop endothelium alterations due to ischemia.<sup>20</sup> Consequently, this can result in thrombolytic treatment failure<sup>20</sup> or poor graft patency. On the other hand, patients with distal autologous vein “bypasses” often present with more advanced atherosclerotic changes than those with short proximal synthetic bypass grafts or those with native arterial thrombotic events. It is worth noting that the synthetic bypass grafts which, in our work, appeared to have a protective influence on limb salvage, can often be reopened with thrombolysis.<sup>5</sup> Undoubtedly, obtaining adequate perfusion can help preserve a viable limb for the time being. Nonetheless, the development of a thin prothrombotic layer in synthetic grafts can affect their patency,<sup>5,20</sup> requiring additional endovascular or open

procedures in order to maintain sufficient distal perfusion. In our cohort, 122 patients underwent a total of 190 additional procedures within a 10-year period after the CDT.

The long-term patency after initial CDT was largely unfavorable. Furthermore, roughly 30% of the patients treated were alive at 10 years post-procedurally. Even though the results are generally in line with the previously reported findings,<sup>7–9,14</sup> the studies are difficult to compare due to dissimilar end point selection. In our work, native arteries showed significantly better primary and assisted primary patency rates in comparison to bypass grafts after the initial treatment with CDT. Patients with bypass grafts usually present with more advanced stages of atherosclerosis that consequently predispose them to an inferior outcome. The secondary patency rates were decreased in both native arteries and bypass grafts. There was no significant difference between them. This adverse outcome is supported by the fact that the long-term amputation-free survival rate was poor as well. The patients presented were





**Fig. 3.** Secondary patency after CDT for native arterial (*black curve*) or bypass graft (*gray curve*) thrombolysis. Kaplan-Meier survival analysis ( $P = 0.88$ ).

predominantly elderly individuals with multiple comorbidities. It is clear that multiple additional procedures, the progression of underlying atherosclerotic changes, diabetic angiopathy, and the loss of runoff vessels and collaterals could eventually result in unfavorable long-term patency rates. It remains debatable whether initial endovascular thrombectomy and additional consequent thrombolysis could have improved the results.

Our study has some limitations. Although most of the data were collected prospectively, it did not include evidence of anti-coagulant treatment for all the patients included in the study. We do not believe, however, that it would have brought any additional and reliable value to our work. The anti-coagulant used in the majority of cases was warfarin, which makes it difficult to determine whether the patient had proper anti-coagulation status at the time of the thromboembolic event. It must be emphasized that, although the events were classified as thrombotic, embolic events could have been present as well. A further diagnostic definition was sometimes impossible and could,

therefore, be misleading especially in regard to statistical analysis.

Another drawback of our study is the fact that some missing data were also obtained retrospectively, which, in turn, could increase bias resulting from miscoding and a lack of clinical information in the patients' files. However, the proportion of the retrospective data was not considerable, and we postulate that it did not influence the results significantly.<sup>21</sup> One more limitation of the study is the inability to perform further subgroup analyses due to the cohort size.

## CONCLUSION

The long-term patency after CDT is unfavorable, and additional procedures are needed to preserve adequate distal perfusion. Approximately 30% of the patients are alive at 10 years after the initial CDT. Increasing age and AF have a negative effect on the patients' survival.

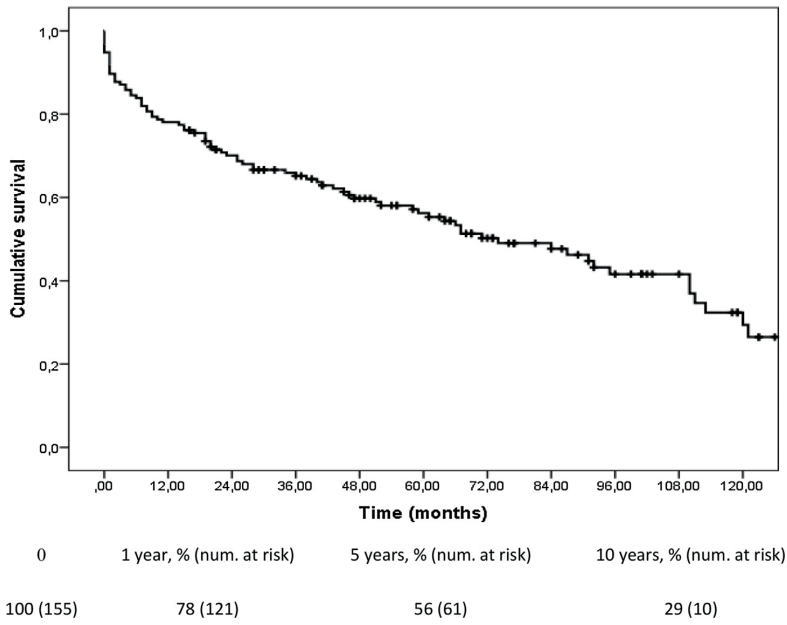


Fig. 4. Cumulative survival of patients with ALLI after CDT. Kaplan-Meier survival analysis.

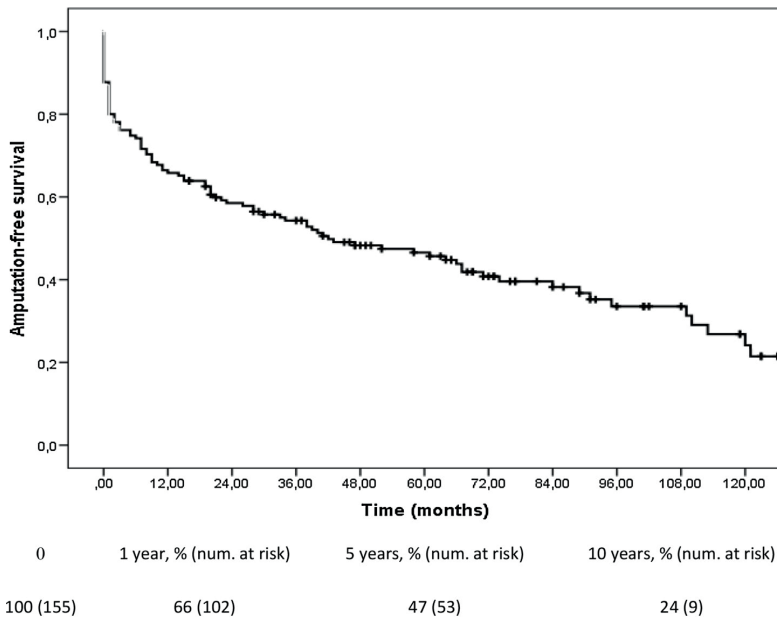


Fig. 5. Amputation-free survival of patients with ALLI after CDT. Kaplan-Meier survival analysis.



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# PUBLICATION IV

**Prognostic risk factors for recurrent acute lower limb ischemia in patients treated with intra-arterial thrombolysis**

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# Prognostic risk factors for recurrent acute lower limb ischemia in patients treated with intra-arterial thrombolysis

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## ABSTRACT

**Objective:** The objective of this study was to assess factors predisposing patients to recurrent acute lower limb ischemia (RALLI).

**Methods:** Acute lower limb ischemia patients treated with catheter-directed thrombolysis (CDT) at Tampere University Hospital and Turku University Hospital between March 2002 and December 2015 were included. The patients' baseline demographics, comorbidities, and other characteristics were assessed retrospectively. Significant factors revealed by univariable analysis were tested in a multivariable model for associations with RALLI. A patency analysis was performed, and the risks of reocclusion were identified. The limb salvage rates after reocclusion were evaluated.

**Results:** Altogether, 303 consecutive patients with a mean age of 71 years (standard deviation, 11.8 years) were included. Of them, 159 (52.5%) were men. A total of 164 (54.1%) native arterial and 139 (45.9%) bypass graft occlusions were initially treated with CDT. On completion of CDT, 204 additional endovascular or conventional surgical procedures on 203 patients were performed to obtain adequate distal perfusion. During a median follow-up of 40 months (interquartile range, 69 months), 40 (24.4%) cases of RALLI occurred in native arteries and 90 (64.7%) in bypass graft patients ( $P < .001$ ). In native arteries, the absence of appropriate anticoagulant and antiplatelet medication was independently associated with the development of acute reocclusions (hazard ratio, 6.51) in the Cox multivariable regression analysis. The patency rates were 86.6%, 72.2%, and 68.0% at 1 year, 5 years, and 9 years, respectively. In bypass grafts, worsened tibial runoff (crural index III; hazard ratio, 2.40) was independently associated with RALLI. The respective patency rates were 60.5%, 34.0%, and 29.2% for synthetic conduits and 30.8%, 20.5%, and 13.7% for autologous vein grafts at 1 year, 5 years, and 9 years. Altogether, 38 (29.2%) major amputations were performed on patients with reocclusions. Patients with synthetic conduits demonstrated superior limb salvage rates after reocclusion in comparison to native arteries or vein grafts ( $P = .025$ ).

**Conclusions:** Appropriate post-thrombolytic antiplatelet or anticoagulant treatment after native arterial events is of great importance, but additional data are needed to improve treatment algorithms. Adequate outflow in bypass graft patients is crucial. Patients with prosthetic bypass grafts have superior limb salvage rates after reocclusion. (*J Vasc Surg* 2019;■:1-8.)

**Keywords:** Acute lower limb ischemia; Thrombolysis; Rethrombosis; Reocclusion; Recurrent acute lower limb ischemia

For >20 years, catheter-directed thrombolysis (CDT) has been used for the treatment of acute lower limb ischemia. The first publications on the use of thrombolysis date to the 1950s.<sup>1</sup> According to the available literature, the method was first introduced in the United

States, but work on the development of fibrinolysis has been carried out in other countries as well.<sup>2,3</sup>

Although the technical success rate of the method exceeds 70% and the short-term amputation-free survival rates are acceptable,<sup>4-6</sup> the postprocedural outcome is often influenced by reocclusions, which results in low long-term post-thrombolytic patency rates.<sup>7,8</sup> As a result, approximately one-quarter of the treated patients undergo major amputations at some point, and the long-term amputation-free survival rates remain disappointing.<sup>7</sup> Despite the introduction of newer thrombolytic agents and advanced infusion methods, the results have not considerably improved<sup>9</sup> and in fact are comparable to those of the urokinase era.<sup>8</sup> This suggests that the development of reocclusions at a later stage could be associated with either impaired hemorheologic blood properties due to inadequate postprocedural antiplatelet and anticoagulant therapy or hemodynamic changes due to physical reasons or organic failure.

Despite the existence of thrombolysis for decades, the role of insufficient runoff in postprocedural patency remains debatable.<sup>4,6,10-12</sup> Existing reports on other factors

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that affect reocclusion development are few, and the data are also inconsistent.<sup>4,6,13,14</sup>

To address these issues, this study aimed to identify specific patient determinants associated with the thrombotic or embolic development of recurrent acute lower limb ischemia (RALLI).

## METHODS

**Data collection.** This is a retrospective study based on a prospectively maintained vascular registry. The registry data along with the patients' files were retrospectively assessed by D.V. and E.S. at Tampere University Hospital (Tays) and by H.H. at Turku University Hospital (TYKS). The angiograms were initially assessed by the radiologists and later by D.V. at Tays and H.H. at TYKS.

A total of 303 consecutive patients who presented with Rutherford category I to IIb<sup>15</sup> symptoms and signs of acute or acute-on-chronic lower limb occlusions of native arteries or bypass grafts were included in the study. The patients were treated with intra-arterial thrombolysis in two tertiary vascular referral centers, Tays and TYKS, between March 2002 and December 2015. The patients were included in the study on an intention-to-treat basis.

Patients with a deep, progressive sensorimotor deficit at admission or contraindications to thrombolysis were treated with conventional surgical modalities and excluded from further analysis. The duration of symptoms no longer than 30 days was generally accepted as the maximum time threshold for CDT initiation.

The Ethics Committees of both hospitals approved the study. No informed consent was required from the patients.

**Thrombolysis protocol and antiplatelet or anticoagulant treatment.** The procedures were performed under local anesthesia either by using a contralateral approach or, alternatively, through the ipsilateral femoral artery. A multihole Cragg-McNamara infusion catheter (Medtronic, Minneapolis, Minn) was placed in the thrombus, and recombinant tissue plasminogen activator (Actilyse; Boehringer Ingelheim, Stockholm, Sweden) was administered as an initial 4-mg bolus. The treatment continued with an infusion of 0.5 mg/h lasting for a maximum of 48 hours, with control angiograms taken every 24 ( $\pm$ 2) hours. For the patients in whom CDT was begun at the end of the day, the first control angiography study was performed earlier. The end points of lytic therapy were complete lysis and flow restoration. Duplex ultrasound scanning and plain radiography could be used for the differential diagnosis of residual thrombus during CDT.

The patients remained in either an intensive care unit or a 24-hour observation ward until the thrombolytic treatment was completed. Blood test results were monitored at least twice daily (complete blood count and potassium, sodium, creatinine, creatine kinase, and myoglobin

## ARTICLE HIGHLIGHTS

- **Type of Research:** Two-center retrospective study of prospectively collected registry data
- **Key Findings:** Recurrent acute ischemia in 303 patients was associated with the absence of anticoagulant or antiplatelet treatment in native arteries and poor runoff in bypass grafts. Limb salvage rates after reocclusion were superior in patients with synthetic grafts.
- **Take Home Message:** Adequate anticoagulant or antiplatelet treatment and sufficient outflow are important determinants of primary patency of revascularizations for acute ischemia. Autologous vein graft rethrombosis is associated with a higher risk of amputations in comparison with prosthetic conduits.

levels). Additional tests were performed as necessary. Fibrinogen levels were not routinely monitored. Necessary endovascular (percutaneous transluminal angioplasty [PTA] or stenting) or conventional surgical procedures (eg, endarterectomy, patch plasty, short-segment jump bypass) were performed on completion of thrombolysis to restore patency of a vessel or bypass graft and to achieve adequate distal perfusion.

Heparin was not infused through the sheath. The low-molecular-weight heparin enoxaparin sodium (Klexane; Sanofi-Aventis, Maisons-Alfort, France) 40 mg or dalteparin sodium (Fragmin; Pfizer, Puurs, Belgium) 5000 IU was administered subcutaneously twice daily instead. Treatment duration with low-molecular-weight heparin ranged from several days to a maximum of several weeks (data not shown) and depended on the patient's level of physical activity and the need for continuous oral anticoagulant therapy. Aspirin, 100 mg daily, was administered to the patients with underlying atherosclerotic changes that had possibly led to thrombotic occlusions. Clopidogrel, 75 mg daily, or its combination with aspirin was administered postprocedurally for a minimum of 1 month to those who underwent PTA of the femoropopliteal segment. Treatment duration was extended to a minimum of 3 months after PTA or prosthetic bypass grafting involving a tibial segment. Warfarin or novel analogues were predominantly administered for varying periods or permanently in patients with cardiac thrombus, hypercoagulable state, atrial fibrillation, and occlusions of unknown origin. In bypass graft patients, severely impaired outflow (crural index III and IV) could become an indication for long-term anticoagulation. In such cases, the decision was made on an individual basis. Combinations of these medications were also prescribed in selected cases.

**Follow-up and reocclusions.** The follow-up was determined as the period between the primary treatment with CDT and the date of study completion or the date of major amputation or death if either of these occurred earlier. Hereafter, the term *reocclusion* is used to refer to an acute reocclusion of lower limb arteries or bypass grafts that is of thrombotic or embolic origin. Thromboembolic events of the same vascular segment and definitely of the same vessel or bypass graft were considered.

The patients were observed for varying periods. Most patients underwent the first routine follow-up visit at 1 month postprocedurally. Subsequent follow-up visits were scheduled at different time intervals until the patients did not require additional manipulations and there were no symptoms and signs of critical limb ischemia. In further post-thrombolytic follow-up, native arterial and proximal bypass graft patients (above-knee) underwent physical examinations in their regional primary health care centers once a year or more frequently. Ultrasound and ankle-brachial index (ABI) measurements were performed on symptomatic patients. Below-knee bypass graft patients underwent hospital follow-up biannually. The visits routinely included physical examinations, ABI, toe pressure measurements, and ultrasound. Additional elective, urgent, and emergency follow-up visits and admissions were arranged on the basis of the clinical situation, and the need for angiography was assessed.

**Definition of the crural index.** Outflow sufficiency after initial CDT was assessed by means of a crural index.<sup>16,17</sup> A previous study had suggested the method as an indicator of survival in patients with symptomatic peripheral artery disease.<sup>15</sup> A high index was shown to be associated with poor amputation-free survival.<sup>16</sup> For this study, the method was chosen because of the simplicity of tibial runoff measurement.

Each tibial vessel is given a score, as follows: 0, no detectable occlusion or minor stenosis; 1, total occlusion of <5 cm; 2, total occlusion of <10 cm; 3, occlusion of <15 cm; and 4, occlusion of >15 cm. The crural index is the sum of these scores for all three tibial vessels. If the sum is 0, the index is 0; for sums of 1 to 3, the index is I; for sums of 4 to 6, the index is II; for sums of 7 to 9, the index is III; and for sums of 10 to 12, the index is IV.<sup>16</sup>

**End points.** The end points of the study were reocclusion events after initial treatment with CDT and limb salvage. The factors affecting the development of reocclusion after CDT were established separately for the subgroups of patients with native arterial occlusions and of patients with bypass grafts.

**Statistical analysis.** The statistical analyses were performed with IBM SPSS Statistics for Macintosh, version 25.0 (IBM Corp, Armonk, NY). The  $\chi^2$  test was used to compare categorical variables; the independent samples

t-test and Mann-Whitney *U* test were applied for continuous variables. Bypass graft patients were assessed as an entity in the risk factor analysis because of the relatively small number of vein conduit patients. Univariable analysis was performed using a Cox proportional hazards model to determine possible risk factors for reocclusion. The parameters were assessed for a proportional hazards assumption with a log-minus-log plot. It was met for all factors tested. The significant parameters demonstrating a *P* value of <.05 were then included in the Cox multivariable backward stepwise regression model. The probability for a stepwise model was set to .05 for entry and to .10 for removal. Kaplan-Meier survival analysis was used to assess the primary patency (freedom from reocclusion) and limb salvage rates of patients with native arterial and bypass graft reocclusion events. A standard error of <10% was considered reliable.

## RESULTS

Altogether, 303 (152 from TYKS and 151 from Tays) consecutive patients treated with CDT for RALLI were included in the study. The majority of the parameter distributions were similar among the patients of both hospitals. The patients' characteristics are presented in Table 1.

Thrombolytic therapy was continued for a median of 1 day (interquartile range [IQR], 1 day; mean, 1.3 days [standard deviation, 0.5 day]). There were no statistically significant treatment duration differences between thrombosis and embolism or native arteries and bypass grafts. The median amount of recombinant tissue plasminogen activator per treatment was 17.0 mg (IQR, 11.0 mg; mean, 20.1 mg [standard deviation, 5.8 mg]).

CDT as a monotherapy was sufficient in 100 cases. One patient underwent endovascular mechanical thrombectomy with a Rotarex system (Straub Medical AG, Wangs, Switzerland) as an adjunct. A total of 204 additional procedures in 203 patients were required on the completion of thrombolysis to restore adequate distal perfusion. Of them, 112 procedures were performed after native arterial occlusions and 92 after bypass graft events. The majority of these (183) were PTAs and stent applications; additional endarterectomies were needed in 13 cases. Eight bypass or jump bypass reconstructions were performed to obtain sufficient distal circulation in the limb. These numbers include 45 cases of partially completed thrombolysis and additional open or endoluminal aspiration thrombectomies for the progression of ischemia during lytic therapy. A failure to dissolve the thrombus in occluded native arteries, bypass conduits, or runoff vessels resulted in four (1.3%) early major amputations performed within 10 days from the initial CDT.

A total of 18 (5.9%) patients developed bleeding complications at the access site and required termination of thrombolysis and blood transfusion. In addition, four patients (1.3%) developed intracranial bleeding, and two

**Table I.** Patients' characteristics

Variables	Native arteries (n = 164)	Bypass grafts (n = 139)	Total (N = 303)
Baseline demographics			
Sex, male <sup>a</sup>	81 (49.4)	78 (56.1)	159 (52.3)
<b>Age, years, mean<sup>b</sup></b>	74 (12.4)	68 (10.2)	71 (11.8)
Comorbidities <sup>a</sup>			
Diabetes	44 (26.8)	30 (21.6)	74 (24.4)
Dyslipidemia	55 (33.5)	61 (43.9)	116 (38.3)
Arterial hypertension	122 (74.4)	101 (72.7)	223 (73.6)
Coronary heart disease	67 (40.9)	50 (36.0)	117 (38.6)
Cerebrovascular disease	23 (14.0)	20 (14.4)	43 (14.2)
Pulmonary insufficiency	27 (16.5)	24 (17.3)	51 (16.8)
History of smoking in last 5 years	34 (20.7)	31 (22.3)	65 (21.5)
<b>Renal insufficiency</b>	15 (9.1)	4 (2.9)	19 (6.3)
<b>Atrial fibrillation</b>	77 (47.0)	40 (28.8)	117 (38.6)
<b>Heart failure</b>	43 (26.2)	19 (13.7)	62 (20.5)
Preprocedural data			
Duration of ischemia, days, median <sup>c</sup>	2 (6)	3 (6)	2 (6)
<b>Type of occluded vessel<sup>a</sup></b>			
Native artery	164 (100)	N/A	164 (53.9)
Synthetic conduit	N/A	113 (81.3)	113 (37.2)
Autologous vein graft	N/A	26 (18.7)	26 (8.6)
Ischemia, Rutherford category <sup>a</sup>			
I	30 (18.3)	29 (20.9)	59 (19.5)
Ila	76 (46.3)	54 (38.8)	130 (43.0)
Iib	58 (35.4)	56 (40.3)	114 (37.6)
Proximal anatomic segment involved <sup>a</sup>			
Iliac	22 (13.4)	16 (11.5)	38 (12.5)
Femoropopliteal	112 (68.3)	105 (75.5)	217 (71.4)
Tibial	30 (18.3)	18 (12.9)	48 (15.8)
<b>Embolitic events<sup>a</sup></b> (valid >99%)	64 (39.0)	5 (3.6)	69 (22.8)
Postprocedural data			
Additional procedure on completion of CDT <sup>a</sup>	112 (68.3)	92 (66.2)	204 (67.3)
Medication <sup>a</sup>			
Aspirin (valid >99%)	37 (22.6)	20 (14.3)	57 (18.8)

(Continued)

**Table I.** Continued.

Variables	Native arteries (n = 164)	Bypass grafts (n = 139)	Total (N = 303)
Clopidogrel (valid >99%)	18 (11.0)	3 (2.1)	21 (6.9)
Dual therapy	27 (16.5)	13 (9.3)	40 (13.2)
Anticoagulant (valid >95%)	77 (47.0)	65 (46.8)	142 (46.9)
Anticoagulant and aspirin	10 (6.1)	10 (7.2)	20 (6.6)
Anticoagulant and clopidogrel	3 (1.8)	5 (3.6)	8 (2.6)
Triple therapy	0 (0)	2 (1.4)	2 (.7)
No medication	11 (6.7)	5 (3.6)	16 (5.3)
Crural index <sup>a</sup>			
0	14 (8.5)	16 (11.5)	30 (9.9)
I	16 (9.8)	20 (14.4)	36 (11.9)
II	45 (27.4)	34 (24.5)	79 (26.1)
III	61 (37.2)	50 (36.0)	111 (36.7)
IV	28 (17.1)	19 (13.7)	47 (15.5)
ABI, median <sup>c</sup> (valid 57%)	0.86 (0.45)	0.93 (0.32)	0.92 (0.39)
TP, mm Hg, median <sup>c</sup> (valid 52%)	70.5 (46.0)	72.0 (57.0)	71.5 (54.0)
Reocclusions			
<b>Reocclusion events<sup>a</sup></b>	40 (24.4)	90 (64.7)	130 (42.9)

ABI, Ankle-brachial index; CDT, catheter-directed thrombolysis; N/A, not applicable; TP, toe pressure.  
 Values are reported as number (%) unless otherwise indicated. Mean data are presented with standard deviation. Median data are presented with interquartile range. The parameters with missing data are presented with valid percentages. Anticoagulant: warfarin or novel analogues. Dual therapy: aspirin and clopidogrel. Triple therapy: anticoagulant, aspirin, and clopidogrel. Statistically significant differences between the variables ( $P < .05$ ) are presented in bold.  
<sup>a</sup>Pearson  $\chi^2$  test.  
<sup>b</sup>Independent samples t-test.  
<sup>c</sup>Mann-Whitney  $U$  test.

(0.7%) of them died. A compartment syndrome was revealed in four (1.3%) cases. Lysis had to be terminated, and adjunct open thrombectomy and fasciotomy were performed on one of these patients. The three other patients did not require thrombolysis termination because the compartment syndrome developed either right after the treatment or at the end of it. The presence of procedural complications had no statistically significant effect on the long-term outcome.

A total of 130 (42.9%) acute reocclusion events of thromboembolic origin were identified during a median study period of 40 months (IQR, 69 months). Reocclusion events predominantly occurred in patients with bypass grafts. The patients with native arterial events were older and more likely to have a history of chronic renal insufficiency, atrial fibrillation, and heart failure. No statistically significant differences were identified between the groups in patient distribution over the anatomic



segments involved, categories of ischemia, tibial outflow indices, or other objective parameters, including the postprocedural ABI or toe pressure (Table I).

### Reocclusions of native arteries

A total of 40 (24.4%) RALLI events were identified among the 164 CDT procedures performed for the treatment of acute native arterial occlusions.

**Risk factor analysis.** On univariable analysis, female sex, poor runoff (crural index IV), and absence of anticoagulant or antiplatelet medication were significantly associated with RALLI.

On multivariable analysis, the absence of anticoagulant or antiplatelet medication was the only independent factor that was associated with the development of reocclusions (Table II). Poor runoff (crural index IV) demonstrated statistical near significance ( $P = .051$ ).

### Bypass graft reocclusions

A total of 90 (64.7%) reocclusion events were identified in 139 patients with bypass grafts of the lower limbs.

**Risk factor analysis.** On univariable analysis, the type of an occluded bypass graft (vein graft) and impaired tibial runoff (crural index III and IV) were significantly associated with the development of rethrombosis. The history of an additional procedure on the completion of CDT had a protective impact.

On multivariable analysis, worsened runoff (crural index III) was the only significant and independent parameter found to be associated with the development of reocclusions (Table II).

### Primary patency

Analysis of primary patency in native arterial and bypass graft patients is presented in Fig 1. Patients treated for acute native arterial occlusions had significantly more favorable rates than those with bypass conduits (Fig 1). The autologous vein bypass graft patients were found to have the lowest patency rates in comparison to the native arterial or synthetic conduit groups (Fig 1).

The primary patency rate in native arteries after embolic events was slightly superior to the corresponding rate after thrombotic events, although the difference was not statistically significant. The prevalence of embolic events in bypass conduits was inadequate to perform a comparison.

### RALLI and limb salvage

A total of 38 (29.2%) major amputations were performed on patients with reocclusions. The majority of the amputations (20 [52.6%]) were performed within the first month after the RALLI (total median time to amputation, 0 months; IQR, 15 months).

In patients who developed RALLI, the limb salvage rate after thrombotic events in native arteries was inferior to the corresponding rate after embolic events. The difference was, however, insignificant. The presence of

**Table II.** Multivariable Cox backward stepwise regression analysis of the factors associated with reocclusion

Type of affected vessel	Hazard ratio (95% CI)
Native arteries	
No medication	6.51 (2.35-18.03)**
Bypass grafts	
Crural index III	2.40 (1.15-5.00)*

CI, Confidence interval.  
Significance levels: \* $P < .05$ , \*\* $P < .001$ .

reoccluded synthetic conduits was associated with superior limb salvage in comparison to thrombosis events in native arterial or autologous vein bypass grafts (Fig 2). An analysis of the role of embolism on the type of vessel could not be performed because bypass grafts were predominantly affected with thrombosis.

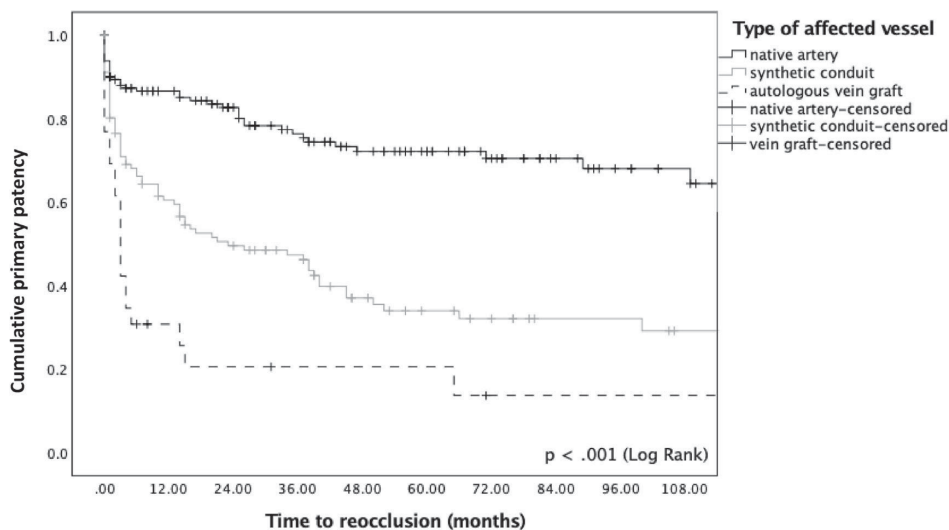
## DISCUSSION

In this study, we have demonstrated that the acute reocclusion rate in lower limb arteries and particularly in bypass grafts was relatively high (42.9%). A failure to maintain appropriate anticoagulant or antiplatelet medical treatment in native arterial occlusions resulted in RALLI. In addition, we found impaired tibial outflow to be significantly and independently associated with the development of rethrombosis in patients with conduits.

Our study has some strengths and limitations. The main strengths include a relatively large number of cases from two vascular centers; homogeneous cohorts because of similar comorbidity distributions among the patients of both hospitals; and long-term follow-up, wide range of factor selection, subgroup analyses, and reduced risk of miscoding and bias because of a limited number of participants processing the data. The main drawbacks include the retrospective design of the study, the presence of several parameters with missing data, and the inability to perform a reliable statistical analysis in regard to the amputation predictors and vein graft occlusions because of the relatively small number of cases and the presence of some incomplete data.

In this series, approximately 40% of all native arterial occlusions were of embolic origin and significantly associated with the presence of atrial fibrillation. The absence of proper anticoagulant or antiplatelet medication was the only independent predictor of RALLI in multivariable analysis. Previous studies<sup>18,19</sup> had suggested the importance of anticoagulation in the prevention of RALLI. This is particularly true for those with cardiac reasons.<sup>18</sup> There are, however, few data available to reject or to support the long-term use of anticoagulants in patients with no atrial fibrillation or intracardiac thrombus.

The use of antiplatelet medication to prevent recurrent atherothrombosis is common. The efficacy of aspirin has been documented in a large number of controlled trials that included thousands of patients.<sup>20</sup> Dual antiplatelet



Limbs at risk:	1 year	3 years	5 years	7 years	9 years
Native artery N=164	114 (86.6)	78 (76.4)	51 (72.2)	30 (70.5)	19 (68.0)
Synthetic conduit N=113	62 (60.5)	41 (47.3)	19 (34.0)	11 (32.1)	8 (29.2)
Vein graft N=26	6 (30.8)	4 (20.5)	3 (20.5)	1 (13.7)	1 (13.7)

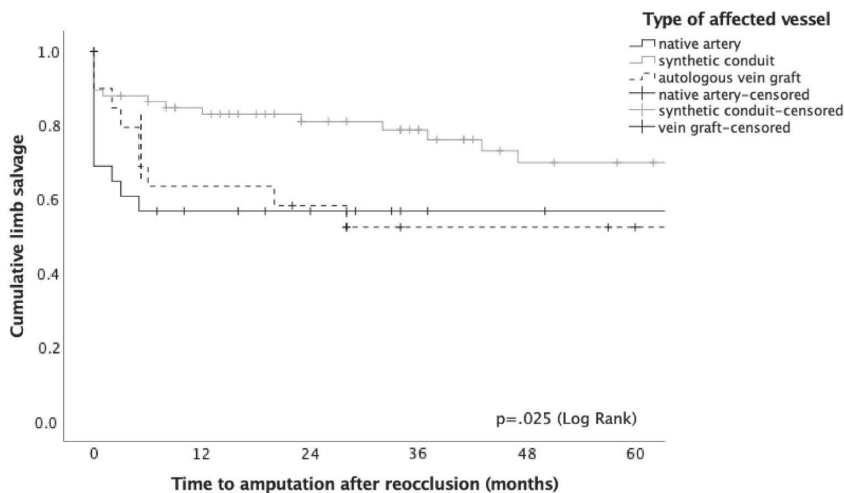
**Fig 1.** Cumulative primary patency—native arteries, synthetic conduits, and vein grafts. Kaplan-Meier survival analysis, 95% confidence interval ( $P < .001$ ). Numbers of limbs at risk and estimated percentage are presented.

therapy also appears to be beneficial in reducing post-procedural complications in patients with peripheral artery disease.<sup>21,22</sup> In addition, data from a recent trial suggested that a combination of rivaroxaban with aspirin for secondary cardiovascular prevention is even superior to rivaroxaban alone or aspirin alone.<sup>23</sup> In this retrospective series, we found that the absence of antiplatelet or anticoagulant medication is independently associated with the development of reocclusions of native arteries. However, we could not demonstrate statistically significant benefits of the administered antiplatelet or anticoagulant therapy on the outcome. There could be several explanations for this phenomenon. First, we are not convinced of sufficient efficacy of warfarin in some patients because of the difficulties in maintaining international normalized ratio within the target range.<sup>24</sup> Second, although the ongoing medication was generally reported, some data were missing. Furthermore, we are confident that there could be patients with suboptimal adherence in taking prescribed drugs. Thus, the group of patients with no appropriate medication could actually be larger and could affect the statistical results. Although we cannot currently draw any final conclusions on the postprocedural antiplatelet or anticoagulant

treatment algorithms, it is clear that the absence of such medication could be decisive. Additional information from a prospective randomized controlled trial would be beneficial.

Bypass graft reocclusions occurred significantly more often than native arterial events. According to our data, impaired tibial outflow was found to be an independent predictor of recurrent graft thrombosis. Little has been published on the role of insufficient runoff in the patency of conduits after treatment with CDT, and the existing reports are difficult to compare because of their heterogeneity. Nackman et al<sup>14</sup> suggested that the identification of occlusive disease in the outflow arteries could predict conduit failure. Taha et al<sup>6</sup> did not find the number of pedal outflow vessels to be significantly associated with the loss of patency in a cohort of native arteries and bypass grafts. On the contrary, a higher preprocedural Society for Vascular Surgery runoff score was associated with a risk of loss of primary patency in the study by Byrne et al.<sup>4</sup>

The primary patency rates were superior in patients with native arterial events compared with their counterparts with synthetic or vein grafts. It has been suggested that “even with adjunctive therapy, vein graft thrombolysis is unlikely to yield durable patency overall.”<sup>14</sup> The primary



Limbs at risk:	1 year	3 years	5 years
Native arterial reocclusion N=29	12 (56.8)	5 (56.8)	3 (56.3)
Synthetic conduit reocclusion N=67	48 (83.0)	30 (78.7)	20 (69.9)
Vein graft reocclusion N=20	12 (63.5)	6 (52.4)	4 (52.4)

**Fig 2.** Limb salvage after reocclusion—native arteries, synthetic conduits, and vein grafts. Kaplan-Meier survival analysis, 95% confidence interval ( $P = .025$ ). Number of limbs at risk and estimated percentage are presented. The vertical dashed line indicates the point from which the number of remaining vein graft cases is small (standard error >10%).

patency rate after graft or stent occlusion was inferior to the primary patency rate after arterial thrombosis or embolus, as reported by Grip et al.<sup>25</sup> Furthermore, in our previous work, we confirmed that the primary and assisted primary patency rates after treatment of native arterial events were superior to those after bypass graft thrombosis.<sup>7</sup> It could be hypothesized that owing to the presence of vasa vasorum in the arterial wall, the intima is obviously not exposed to the same degree of ischemic damage as the vein bypass graft walls are, thus partially explaining our findings. Occluded bypass grafts can generally be reopened with CDT, but long-term prognosis remains inadequate.<sup>7,13,26</sup> Endothelial damage in vein grafts and the development of a thrombogenic layer in synthetic conduits<sup>27</sup> could be a consequence of thrombosis, leading to a poor postprocedural outcome.

The limb salvage rate in patients with synthetic conduits was superior to the rate in individuals with autologous vein grafts and native arterial recurrent events. The information to address this issue is limited.<sup>4,6,14,25</sup> Unfortunately, none of the mentioned works evaluated limb salvage from the viewpoint of RALLI. Therefore, the comparison of these data to our results would be unjustified.

Almost all of the patients with native arterial recurrent events had signs of considerably diminished outflow. Subsequently, the inability to restore sufficient circulation resulted in major amputations. Irreversible ischemic

changes of the endothelium in vein bypass grafts could expose such conduits to poor patency rates. Furthermore, at our institutions, autologous vein grafts are commonly used for distal (below-knee) bypass surgery. Advancing distal peripheral artery disease and an inability to restore circulation in the limb after graft failure would obviously also predispose many of these patients to major amputations. In contrast, synthetic conduits are often used for the correction of more proximal lesions. Although the patency of reopened conduits is usually poor, the limb can generally be salvaged by means of additional surgical procedures (eg, additional bypass grafting involving more distal segments), thus explaining our results.

## CONCLUSIONS

Appropriate post-thrombolytic antiplatelet or anticoagulant treatment after native arterial events is of great importance, but additional data are needed to improve the treatment algorithms. Adequate outflow in bypass graft patients is crucial. Patients with prosthetic bypass grafts have superior limb salvage rates after reocclusion.

## AUTHOR CONTRIBUTIONS

Conception and design: DV, HH, NO, VS  
Analysis and interpretation: DV, HH  
Data collection: DV, HH, ES

Writing the article: DV

Critical revision of the article: DV, HH, ES, NO, VS

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