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DISSERTATION

‘‘Anastomotic engineering in above-knee femoropopliteal bypass with pre-cuffed distal end. Clinical results of a multi-centre cohort study and investigation of in vivo hemodynamics.’’

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Introduction

1.1. Peripheral arterial disease: Aetiology, pathology and clinical principles

Peripheral arterial disease (PAD) comprises those entities, which result in obstruction of blood flow to the arteries, exclusive of the coronary and intracranial vessels. The term PAD is primarily used to describe chronic arterial occlusive disease in the arteries of the lower extremity.

Intermittent claudication is the earliest and most frequent symptom of PAD followed by pain at rest during the night and relieved by limb dependency. Typical pain localization for claudication is in the calf and thigh, while ischemic pain at rest is mostly localized in the foot. Further progression of PAD leads to ischemic ulceration and gangrene[1,2]. The most common classification systems of PAD are the Fontaine classification[3] and the Rutherford[4] classification (Table 1).

Table 1: Stages of peripheral arterial disease according to the Fontaine and Rutherford classifications

Fontaine		**Rutherford**	
Stage	Clinical	Grade	Clinical
I	Asymptomatic	0	Asymptomatic
IIa	Mild claudication	1	Mild claudication
IIb	Moderate to severe claudication	2	Moderate claudication
		3	Severe claudication
III	Ischemic rest pain	4	Ischemic rest pain
IV	Ulceration or gangrene	5	Minor tissue loss
		6	Major tissue loss

Disease prevalence for asymptomatic disease based on objective testing has been evaluated in several epidemiologic studies and is in the range of 3% to 10%, increasing to 15% to 20% in persons over 70 years[2,5].

The prevalence of intermittent claudication increases from about 3% in patients aged 40 years to 6% in patients aged 60 years[2].

A variety of risk factors has been identified for PAD, which are almost identical to those of atherosclerotic disease. The most important of these are age and sex with elderly men having a higher risk of developing atherosclerosis of the lower extremities. Hypertension causes a 2.5-fold increase in the risk of PAD in men and a 3.9-fold increase in women. Diabetes mellitus is a further important risk factor for large vessel atherosclerotic occlusive disease. PAD is also closely linked to smoking. The Framingham study showed the risk of PAD in smokers is twice that in non-smokers[6]. Further risk factors include dyslipidemia, hyperhomocysteinemia, chronic renal insufficiency and race[1,2,7].

1.2. Natural history of PAD

The natural history of chronic PAD is strongly related to the stage of disease. About 75% of patients with intermittent claudication show no disease progression and the risk of major amputation within 5 years is estimated to be approximately 2%. Deterioration of disease is more likely to occur within the first year after diagnosis (7-9%). The annual rate of clinical deterioration thereafter is 2-3%[1,2,8].

In patients with critical limb ischemia, it is not possible to make a reliable statement concerning the natural history of PAD since the vast majority of these patients undergo endovascular or conventional vascular surgery.

1.3. Historic review of vascular surgery for PAD

The first bypass procedure is attributed to Ernst Jeger. In his book „Die Chirurgie der Blutgefässe und des Herzens“[9], published in 1913, he describes the exact surgical technique of a bypass operation.

Until the end of the 2nd World War, Leriche and Diez dominated the field of vascular surgery with their work on endarterectomy and lumbar sympathectomy for PAD, respectively. At the beginning of the 20th century the French surgeon Le Jars performed the first vessel desobliteration (thrombectomy of a traumatic thrombosis), but Dos Santos established in 1947 thromboendarterectomy as a feasible procedure in vascular surgery[10]. Vorhees et al.[11] described for the

first time in 1952 the use of tubes constructed from Vinyon N cloth in bridging of arterial defects, thereby starting a new era in vascular surgery.

Polyethylene terephthalate or Dacron and polytetrafluoroethylene (PTFE) are the two most common prosthetic materials used today for bypass grafting. Dacron was developed in Great Britain in 1941 by 2 chemists, Whinfield and Dickinson[12]. In 1952, DeBakey made the first Dacron tube graft for aortic reconstruction on his wife's sewing machine.

PTFE was developed by a DuPont researcher, Roy Plunkett, in 1938, and it was first marketed under the Teflon trademark (DuPont) in 1945. Researchers at W.L. Gore & Associates, Inc (Newark, Del, USA) developed expanded PTFE (ePTFE), which is much more compliant and porous compared to its non-expanded counterpart. Campbell et al. reported the use of ePTFE as a lower-extremity bypass conduit in 1976 for the first time[12,13].

1.4. Current therapeutic options

The management of patients with lower extremity PAD needs to focus on the risk factors important for the progression of generalized atherosclerosis and the interventions such as pharmacotherapy, endovascular therapy, or surgery to remedy the lower extremity symptoms.

1.4.1. Control of risk factors

Patients should undergo basic haematologic and metabolic laboratory assessment. Screening of patients for hypercoagulable syndromes is not cost-efficient, but should be considered for patients with repeated failure of revascularization or young patients. Cessation of smoking, optimization of nutrition and control of risk factors such as hypertension, diabetes mellitus and/or hyperlipidaemia are key-elements in the management of PAD. Antiplatelet agents should be administered, usually aspirin[1,2,8].

1.4.2. Exercise training

A meta-analysis published in the Cochrane Collaboration[14] that considered only randomized controlled trials concluded that exercise improved maximal walking time by an average of 150 percent (range, 74 to 230 percent). Exercise-induced improvement of maximal walking ability exceeded those attained with medication (20 to 25 percent with pentoxifylline and 40 to 60 percent with cilostazol)[15-17].

1.4.3. Pharmacotherapy

Patients with PAD and claudication should be initially treated by conservative measures adjuvant to an exercise-training program. So far no agent has provided sufficient efficacy to gain widespread acceptance. Agents that have been used in the treatment of PAD include vasodilator drugs, such as papaverine, one of the first drugs studied for claudication, pentoxifylline and cilostazol. Pentoxifylline is a methylxanthine derivative that improves the deformability of red cells and white cells, lowers plasma fibrinogen concentrations, and has antiplatelet effects. Cilostazol is a phosphodiesterase type 3 inhibitor which increases intracellular concentrations of cyclic-AMP. Pentoxifylline has limited efficacy but cilostazol improves both pain-free and maximal treadmill walking distance and the quality of life. Other drugs, like naftidrofuryl and levocarnitine, have also been shown to improve pain-free walking distance and maximal walking distance respectively. However, larger randomized controlled studies are needed[1,18].

Intravenous administration of prostaglandins also has been shown to have a positive effect on critical ischemia in several studies and could perhaps represent an effective agent for treating claudication, although no firm data exist at this stage[18,19].

There are now numerous clinical trials demonstrating the beneficial effects of statins on the natural history of atheromatous disease and its treatment.

In the Cholesterol Lowering Atherosclerosis Study, lipid-lowering therapy was associated with stabilization or regression of femoral atherosclerosis[20]. A meta-analysis, performed from randomized trials of lipid-lowering therapy in 698

patients with PAD, demonstrated that lipid- lowering therapy reduced disease progression – as measured by angiography – and examined the severity of claudication[21,22].

In summary, lipid-lowering therapy has been beneficial for patients with PAD[23], who often suffer from coexisting coronary and cerebral arterial disease. The current recommendation for patients with PAD is to achieve a serum LDL cholesterol concentration of less than 100 mg per decilitre (2.6 mmol per liter) and a serum triglyceride concentration of less than 150 mg per deciliter (1.7 mmol per liter)[22].

1.4.4. Revascularization

1.4.4.1. Endovascular therapy

Endovascular therapy has been established over the past years as a secure and feasible alternative in the management of femoropopliteal arterial occlusive disease. Several studies have suggested that percutaneous transluminal angioplasty (PTA) with or without stent implantation should be the primary choice of treatment for chronic limb ischemia when possible[24-26].

Innovative techniques such as subintimal recanalization, cutting balloons, laser-assisted angioplasty, cryoplasty and the implantation of nitinol, drug-eluting or covered stents for the superficial femoral artery are available and feasible in the treatment of superficial femoral artery stenosis or occlusion[24,26-31]. Novel devices such as the SilverHawk™ Plaque Excision System and the Outback® Re-Entry Catheter present, along with other similar devices which have been developed for the same cause, reliable alternatives to standard angioplasty and stent achieving excision of the obstructing arterial plaque using a minimally invasive technique[31,32].

Procedural indications have been liberalized when compared with those for surgical procedures. It has been argued that the minimally invasive nature of percutaneous modalities warrants broadened application. Nevertheless, although devices and results have improved over time, the long-term patency of percutaneous interventions remains inferior to open surgical techniques[33,34].

The risk-benefit ratio associated with endovascular versus open surgical revascularization is a question that can only be answered by well-designed large comparative clinical trials. In patients with anatomically appropriate lesions, however, most practitioners use endovascular interventions preferentially; a practice based on the presumption that it is associated with less risks for the patient.

1.4.4.2. Bypass and choice of conduit

Surgery for femoropopliteal PAD includes mainly angioplasty of the femoral artery with or without patch and the implantation of femoropopliteal bypasses. Furthermore, a novel technique, the Remote Superficial Femoral Artery Endarterectomy (RSFAE) was introduced, which combines open and endovascular surgery for the treatment of chronic long-segment superficial femoral artery occlusive disease. Gisbertz et al.[35] reported in their randomized prospective trial that RSFAE and bypass with alloplastic material perform equally well in terms of primary and secondary patency rates at 1 year follow-up.

In cases of occlusion of the superficial femoral artery, a relevant stenosis of the deep femoral artery has to be treated before proceeding to a bypass operation[36].

A variety of grafts for above-knee reconstructions and multiple modifications on the graft geometry, material configuration and surface have been tested to improve patency rates (Table 2).

Table 2: Peripheral grafts currently commercially available[37]

Material	Company	Product Name	Product Information	Product Uses
ePTFE [-(CF_2-CF_2)-]n	Braun	VascuGraft	Unmodified	Peripheral bypass/hemodialysis access
	W.L. Gore & Associates, Inc	Gore-Tex	Unmodified	Peripheral bypass/hemodialysis access
		Gore-Tex Stretch	Stretch	Peripheral bypass/hemodialysis access
		Gore Intering	Unibody intrawall radially supported	Peripheral bypass/hemodialysis access
		Gore Propaten	Heparin bonded with Carmeda BioActive Surface for reduced thrombogenicity	Peripheral bypass/hemodialysis access
	Bard	Impra CenterFlex	No modification	Peripheral bypass/hemodialysis access
		Impra Carboflo	Carbon-coated, decreased platelet adhesion	Peripheral bypass/hemodialysis access
		Venaflo II	Carbon-coated, cuffed	Hemodialysis access
		Distaflo	Carbon coated, cuffed	Peripheral bypass (below knee)
		Dynaflo	Carbon coated, cuffed	Peripheral bypass (above knee)
	Boston Scientific	Exxcel Soft Vascular Graft	Unmodified	Peripheral bypass/hemodialysis access
		Exxcel Vascular Access Graft	Wrapped with ePTFE to enhance mechanical strength	Peripheral bypass/hemodialysis access
	Angiotech/Edwards Life Sciences	Lifespan	Reinforced	Peripheral bypass/hemodialysis access
	Atrium	Advanta VXT	Soft-Wrap technology makes grafts softer and easier to handle	Peripheral bypass/hemodialysis access
		Advanta SST	Trilaminate construction allows graft to "pulse" after implantation	Peripheral bypass
		Advanta VS	Single layer, improved incorporation after implantation	Peripheral bypass/hemodialysis access
		Flixene	3 Layers laminated with biomaterial film to increase durability; very strong and durable	Peripheral bypass/hemodialysis access
	Vascutek	Maxiflo Ultrathin and Wrap	Unsealed	Peripheral bypass/hemodialysis access
		SealPTFE Ultrathin and Wrap	Gelatin sealed	Peripheral bypass/hemodialysis access
		Taperflo	Gelatin sealed and tapered	Peripheral bypass/hemodialysis access
		Rapidax*	Trilaminar, self-sealing	Hemodialysis access 24 h after placement

(*Continued*)

Table 2: continued

Material	Company	Product Name	Product Information	Product Uses
Poly(ethylene terephthalate) polyester [O-C= -C_6H_6-O-C=O-CH_2CH_2]n	Braun	SilverGraft	Knitted, double velour, silver impregnated	Peripheral bypass with anti-infection protection
		Protegraft	Knitted, double velour	Peripheral bypass
		UniGraft	Woven velour, gelatin impregnated, available in single and double velour	Peripheral bypass
	InterVascular (Distributed in US by W.L. Gore & Associates, Inc)	InterGard Woven and Knitted	Collagen coated woven/knitted polyester for easier handling	Peripheral bypass
		InterGard UltraThin		Peripheral bypass
		InterGard Heparin	Heparin coated, knitted polyester	Peripheral bypass
		InterGard Silver	Antibacterial	Peripheral bypass, with anti-infection protection
	Boston Scientific	Hemashield Gold Microvel	Collagen impregnated, knitted double velour	Peripheral bypass
		Hemashield Platinum	Woven velour	Peripheral bypass
	Atrium	Ultramax	Zero porosity, gel impregnated, knitted velour	Peripheral bypass
	Vascutek	Fluoropassiv	Knitted, gelatin impregnated, fluoropolymer coated	Peripheral bypass
		Gelseal	Knitted, gelatin impregnated	Peripheral bypass
		Gelsoft	Knitted, gelatin impregnated, zero porosity	Peripheral bypass
		GelsoftPlus	Knitted, gelatin impregnated, dilation resistant	Peripheral bypass
		VP1200K	Unsealed version of Gelsoft	Peripheral bypass
Polyurethane [-NH-O=C-O-R-]n	Thoratec (Distributed in US by Impra)	Vectra	Thoralon trilayer (polyetherurethaneurea and a siloxane)	Hemodialysis access 24 h after placement
	LeMaitre	Expedial*	Polycarbonate urethane inner layer, heparin impregnated	Hemodialysis access 24 h after placement
Other	Boston Scientific	Fusion Graft*	Combined ePTFE graft fused to knitted polyester outer layer	Hemodialysis access 72 h after placement

The information included is intended to be representative rather than comprehensive.
*These products are currently available only in clinical trials.

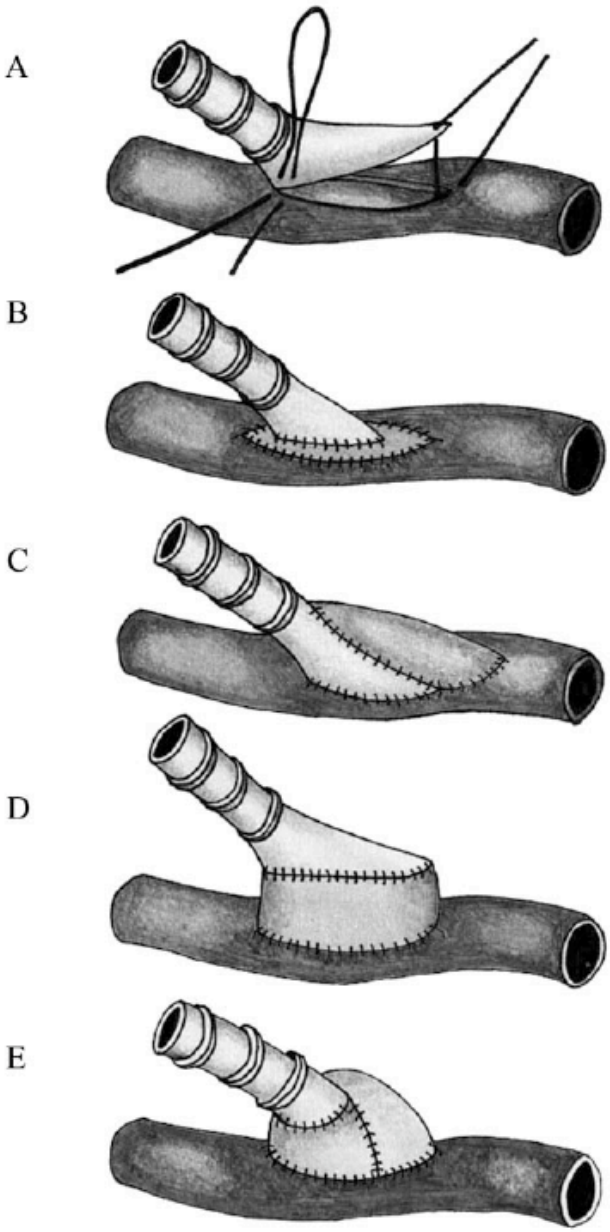
Although the best conduit was disputed for a long time in above-knee femoropopliteal revascularizations, two meta-analyses clearly showed that vein is superior to all other grafts in the long term regarding patency rates and limb salvage[38,39]. Given the relative ease of implantation and the desire to spare the autologous vein for a later use, ePTFE is preferred as the conduit of choice for above-knee revascularizations in many vascular centres. Dacron grafts are a valid and reliable alternative for above-knee arterial bypass[40,41]. Human umbilical vein grafts are reported to achieve better patency rates than Dacron or PTFE[39,42,43] but the difficulty in suturing the anastomosis and the long-term complications such as anastomotic aneurysms as well as the very high

production costs of the graft have not allowed this graft to be widely applied and is no longer commercially available[42,44,45].

Poor patency of prosthetic bypass grafts has led several researchers to concentrate efforts on improving available prosthetic materials. With this objective, a great deal of attention has been focused on surgical modifications, materials and coatings, protein modifications, endothelial cell seeding, and nitric oxide (NO) modifications of existing prosthetic grafts with the intention of improving graft patency and clinical outcomes (Table 2)[37,46].

In an effort to improve the compliance between prosthetic grafts and recipient artery, as well as hemodynamics, the use of vein cuffs or vein patches (Figure 1) has been proposed in order to minimize MIH in the distal anastomosis and consequently achieve better patency rates[47,48].

Figure 1: Surgical techniques for prosthetic graft modifications at the distal anastomosis: A, standard end-to-side anastomosis; B, Linton patch; C, Taylor patch; D, Miller cuff; E, St. Mary's boot (Figure from Kapadia et al.[37])



Continuous research in the field of vascular grafting has led to the introduction of several types of grafts such as the Femoro-Crural Patch Prosthesis (FCPP) or the Distaflo® graft to optimize hemodynamic patterns in the distal anastomosis through modification of the anastomosis geometry or graft distensibility[49,50].

Protein coating or binding proteins to grafts is another approach for improving graft patency. Slow-releasing heparin-bonded grafts have been suggested to achieve acceptable patency rates under the assumption that the immobilized heparin binds antithrombin and therefore retains its anticoagulant properties on the graft surface, thereby improving the thromoboresistance of the graft[42,51,52]. Bosiers et al.[51] reported in a 209-patient randomized study after comparing a non-heparinized with a heparin-bonded ePTFE graft that the performance of the heparin-bonded graft was significantly better, with an overall 1-year patency rate of 70% and rates of 75% and 38%, respectively, for above-knee and below-knee femoropopliteal bypasses.

Another anticoagulant protein is thrombomodulin, an endothelial cell membrane–bound protein that complexes with thrombin and activates the protein C anticoagulation pathway. Other proteins or anticoagulants used include tissue plasminogen activator or hirudin, a direct thrombin antagonist which degrades fibrin complexes[37].

Endothelial cells secrete several substances that inhibit both thrombosis and MIH and are critical for homeostasis and maintenance of vascular integrity in the circulation. Because of their vasoprotective properties, researchers have attempted to modify prosthetic grafts by seeding endothelial cells onto the luminal surface and thereby creating a biologically active graft. In most cases venous endothelial cells were harvested and seeded on the graft surface within a one- or two-stage procedure. Deutsch et al. reported a cumulative primary patency rate of 68% at 5 years for above-knee femoropopliteal bypasses from a series of 318 autologous endothelium-seeded ePTFE grafts[37,46].

More recently, investigators have used endothelial progenitor cells (EPCs) isolated from peripheral blood or bone marrow to seed prosthetic vascular grafts[53]. Other sources of seeding have included microvascular endothelial cells derived from fatty tissue such as subcutaneous fat or omentum. The advantages of these cells include ease of obtaining them in large quantities and obviating the need for cell culture. However, seeding of these cells has not achieved promising results[54]. Prosthetic graft modifications incorporating NO to

improve patency are the subject of current and active focus. NO is produced constitutively from endothelial cells and is vital in the regulation of vascular tone, prevention of platelet aggregation and inhibition of vascular smooth muscle cell proliferation and migration[37,55].

At present the choice of the conduit for peripheral revascularizations is left to the discretion of the surgeon. For many of the currently available grafts no data regarding performance or patency rates exist, especially when compared to each other.

1.5. Hemodynamic patterns in the termino-lateral anastomosis of bypass grafts

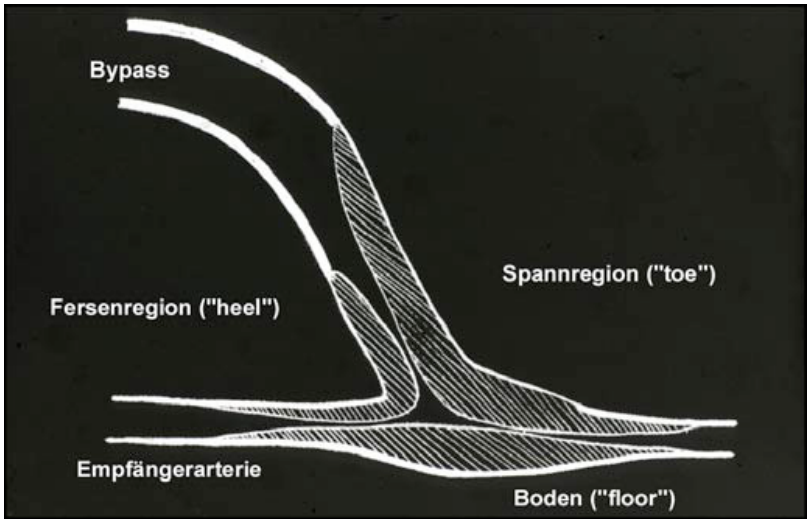
In the human body, no termino-lateral anastomoses naturally exist. These anastomoses are performed during revascularizations to achieve antegrade distal perfusion and retrograde perfusion of the more proximal recipient arterial segment. As a result, suboptimal hemodynamic patterns are found in this non-physiologic anastomosis.

These hemodynamic patterns have been shown to cause stenosis or occlusion at the anastomotic site, mainly due to the formation of myointimal hyperplasia (MIH) in this area.

An extensive body of literature describing effects of shear stress on endothelial cells has contributed to our understanding of the interactions between shear stress and blood vessels. Laminar shear stress is atheroprotective, whereas oscillatory or disturbed shear stress correlates with areas of atherosclerosis and MIH in vivo[56].

The compliance mismatch between vascular graft and native arterial wall has a similar effect on MIH formation. The role of flow stagnation is widely accepted as a risk factor for MIH development due to low wall-shear-stress that is applied at certain areas of the termino-lateral anastomosis. Points subjected to flow stagnation in the anastomosis include the floor, toe and heel of the anastomosis as demonstrated in the figure (Figure 2).

Figure 2: Points subjected to flow stagnation in a terminolateral anastomosis[57]



Several studies have suggested that low shear stress is associated with elevated MIH at the critical locations of the anastomosis[58-60].

1.6. Background and purpose of the study

The pre-cuffed ePTFE prosthesis (Dynaflo®, Bard Peripheral Vascular Inc, Tempe, Ariz) was developed using anastomotic engineering to optimize hemodynamic patterns within the distal anastomosis and thereby to reduce the development of MIH, improving this way patency rates of above-knee femoro-popliteal bypasses[61,62]. This end-to-side anastomosis was shaped analogous to the Distaflo® prosthetic graft, which is a pre-cuffed graft used for below-knee arterial reconstructions[50]. Clinical studies showed non-inferiority of the Distaflo® prosthesis as compared to vein patch and cuff techniques, such as the Miller collar, when used for infragenicular or crural revascularization[50,63]. There are no clinical data concerning the use of the Dynaflo® prosthetic graft. To investigate the performance of this novel graft, a prospective clinical, non-randomized multi-centre cohort study was designed. Moreover, so far the special hemodynamics described for the in vitro situation has been poorly investigated in vivo. We assumed that ultrasound (US) and contrast-enhanced ultrasound (CE-US) might be suitable to visualize flow patterns.

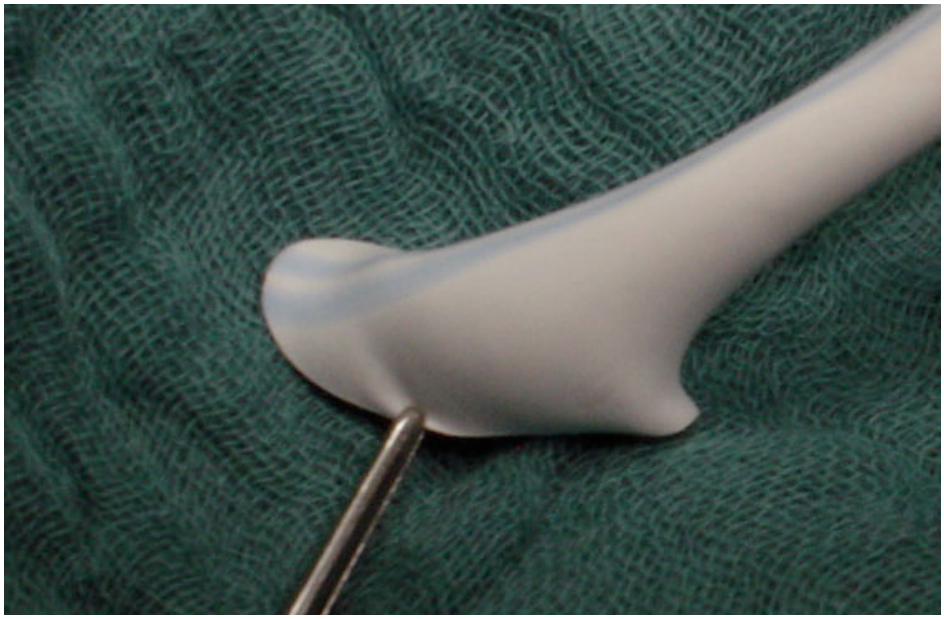
The study included two branches:

1. The clinical study with endpoints primary, primary assisted and secondary patency of the Dynaflo® graft for above-knee femoropopliteal bypass and complications of the operation.
2. The in vivo examination of the hemodynamics within the distal anastomosis of the pre-cuffed graft to determine whether the special hemodynamic patterns that have been demonstrated in vitro can be replicated in vivo.

2. Materials and Methods

The Dynaflo® graft (Figure 3) is available since March 2005 for application in clinical practice. The implantation of the graft began in three certified vascular centres in Germany within a prospective non-randomised cohort study. The Dynaflo® grafts were implanted by all vascular surgeons (n=11) of the participating centers as well as by vascular trainees (n=5) under supervision.
Between March 2005 and August 2007, 135 Dynaflo® grafts were implanted in 134 patients requiring above-knee femoropopliteal bypass for PAD.

Figure 3: Dynaflo® ePTFE graft with the pre-cuffed distal end designed to optimize hemodynamics within the end-to-side anastomosis with the recipient artery.



2.1 Clinical study design

2.1.1. Clinical study inclusion/exclusion criteria

The study included all consecutive adult patients with claudication (Rutherford 2-3 / Fontaine IIb) or chronic critical limb ischemia (Rutherford 4-6 / Fontaine III-IV), who were considered suitable for elective revascularization by use of a supragenicular prosthetic bypass graft, provided that the patients had given written consent to take part in the study and follow-up.

All patients were presented in an interdisciplinary conference with vascular surgeons, angiologists and interventional radiologists, as it is obligatory for certified vascular centers in Germany, to discuss less invasive therapeutic options. The indications for operative treatment with a supragenicular bypass included PAD in stages IIb – IV to the Fontaine classification.

Patients were considered candidates for implantation of a supragenicular bypass after being evaluated with respect to the following points:

- Patients with claudication should have completed exercise training for claudication without sufficient improvement (>50%) and persistent quality of life-limiting claudication.
- No percutaneous transluminal angioplasty with or without stent implantation was feasible or was considered a reasonable alternative with respect to long-term results. To achieve homogeneity in the group of patients undergoing above-knee femoropopliteal bypass between the three centers, no patients with an occlusion length of the superficial femoral artery of less than 15 cm were included before undergoing endovascular approach.
- No pathology (e.g. stenosis >50%) of the deep femoral which could be treated with patch-angioplasty or PTA was present.
- Adequate inflow to the common femoral artery was a prerequisite for inclusion in the study. If not present or if at least improvable, it had to be established before or during the operation. Adequate inflow was considered present, when triphasic flow in the common femoral artery was found using color-coded Doppler sonography or when the iliac vessels were angiographically free of >30% stenosis or multiple stenoses. A non-severely calcified segment of adequate length of the proximal popliteal artery as estimated by the surgeon should be available to assure an exact configuration of the cuff without modification.
- An appropriate distal popliteal artery (< 50% stenosis) and at least one vessel outflow in either the tibial or peroneal vessels were present.

Exclusion criteria were:

- a general inoperability of the patient
- pregnancy
- refusal of the patient to participate in follow-up examinations
- emergency surgery
- short (less than 1 year) life expectancy
- popliteal aneurismal disease and
- extended infection of the affected limb which could put the implanted graft in risk of infection

2.1.2 Preoperative diagnostic modalities

The PAD-specific preoperative work-up of the patients included evaluation of:

1. ankle-brachial index (ABI)
2. Free-of-pain walking distance on the treadmill with 3 km/h speed and 12% inclination for patients with claudication.
3. Conventional digital subtraction angiography (DSA) was the preferred preoperative imaging modality in patients scheduled to undergo surgical treatment but computed tomography angiography (CTA) or magnetic resonance angiography (MRA) were also accepted in selected cases.
4. The inflow to the common femoral artery was assessed either with color-coded Doppler sonography (triphasic flow) when only a fine needle angiography had been performed as preoperative diagnostic modality of the affected limb or angiographically when angiography of the aortoiliac vessels was available.

2.1.3. Postoperative care and follow-up

All patients underwent on postoperative day three to five a control digital subtracted angiography of the operated limb when no intraoperative angiography had been performed. An intraoperative angiography was not obligatory according to the study protocol and the decision was left to the discretion of the surgeon. For two days postoperatively all patients were put on anticoagulation with low

molecular weight heparin at a dosis of 5000 IU of Dalteparin per day. On the third postoperative day, when there were no contraindications such as major wound healing disorder or planned operation, further anticoagulation was initiated with antiplatelet agents or warfarin. Heparin was continued until antiplatelet- or warfarin therapy reached effective levels. The standardized postoperative anticoagulation scheme was:

- 100mg aspirin, or 75mg clopidogrel in cases of aspirin intolerance, daily lifelong when ≥ 2 tibial or peroneal vessels were patent
- Warfarin derivatives were administered on a daily basis to compliant patients with only one patent crural vessel, even when they presented with claudication or after revision for bypass occlusion or thrombus formation at the distal anastomosis and when no contraindications were present (optimal INR ratio 2.5 – 3.5).
- 100mg aspirin daily lifelong and clopidogrel 75mg daily for 6 weeks when a PTA with stent implantation was simultaneously performed with the supragenicular bypass.

When patients were under warfarin derivatives or a combination of aspirin and clopidogrel for another medical indication prior to the revascularization, the anticoagulation scheme was changed only after consulting a cardiologist.

The follow-up protocol included office visits, clinical examination and color-coded Doppler sonography at 3, 6 and 12 months and every 6 months thereafter. The ankle – brachial index was measured on every visit.

In case of complications such as graft occlusion, high risk of graft occlusion or wound infection a standardized pathway of complication management was established. In case of thrombus in the distal anastomosis without graft occlusion, verified on DSA, therapeutic options were considered. Operative revision or change of anticoagulation regimen to warfarin derivatives to prevent embolization or bypass occlusion was only decided depending on the amount and localization of thrombus material.

Cases, where inflow problems were diagnosed on follow-up, were treated with PTA and stent or surgical therapy. In case of acute, sub-acute or chronic graft

occlusion the standard method of treatment was thrombectomy with revision of the distal anastomosis. In selected cases, intra-arterial local rt-PA lysis was considered in the absence of contraindications.

2.1.4. Surgical technique

The popliteal artery was exposed in its first segment by a supragenicular, medial approach. Inspection and palpation confirmed suitability as recipient artery for an above-knee bypass. Exposure of the common femoral as well as the proximal deep femoral artery and the proximal SFA was performed by an infrainguinal incision. The prosthetic graft (Dynaflo®) was implanted extraanatomically or anatomically with the help of a tunnelling instrument, preventing kinking of the graft proximal to the distal cuff. Systemic heparin was administered at a dose of 5,000 IU and the popliteal artery was clamped. After longitudinal arteriotomy the distal anastomosis was performed, using a running suture (Prolene 5-0, Ethicon, Norderstedt, Germany). The length of popliteal arteriotomy was adapted carefully to the requirements of an optimal cuff configuration while suturing. The pre-cuffed end of the graft was not modified in any way. The arteriotomy was performed long enough, so that the whole cuff could be sewn without modification. Nevertheless, the arteriotomy should not be too extensive otherwise the cuff would be stretched on the longitudinal axis. This could compromise the cuff in the transverse axis. No exact length of required popliteal artery was defined in the protocol and the decision concerning the length of the arteriotomy as well as the further technique for the suture of the cuff was left to the discretion of the surgeon. An intraoperative angiogram was obligatory only when a technical intraoperative error was suspected or when a more proximal stenosis (not identified preoperatively) was assumed during the flushing maneuver from the common femoral artery. The proximal anastomosis was performed end-to-side with the common femoral artery. When necessary, adequate inflow was established prior to the bypass by intaoperative balloon angioplasty or retrograde desobliteration of the iliac vessels.

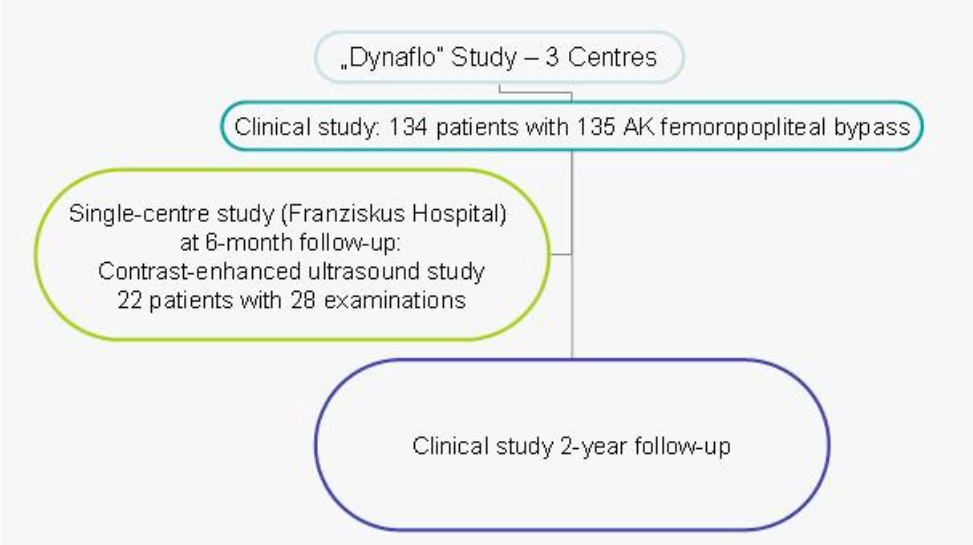
2.2. In vivo hemodynamics in the distal anastomosis

2.2.1. Inclusion and exclusion criteria for the contrast-enhanced ultrasound (CE-US) study

Patients who had undergone implantation of a Dynaflo® graft in one of the three participating vascular centers (Franziskus Krankenhaus, Akademisches Lehrkrankenhaus der Charite, Universitätsmedizin Berlin) as part of the prospective clinical study were considered as candidates to participate in the contrast-enhanced ultrasound (CE-US) study designed to evaluate the in vivo hemodynamics within the distal anastomosis (Figure 4). All patients were asked to sign an additional written consent in order to participate in this study.

Exclusion criteria for the CE-US study were: severe cardiac insufficiency (NYHA III-IV), known allergy to US contrast-agent, and known occlusion of the femoropopliteal bypass. Written informed consent was obtained from all patients.

Figure 4: Study design of the Dynaflo® clinical and contrast-enhanced ultrasound studies.



2.2.2 Imaging techniques

All patients included in the study underwent postoperative fine-needle angiography of the operated leg when no adequate intraoperative angiography was available. Color-coded Doppler ultrasound (CDUS) was performed

according to the study protocol on the 6-month follow-up. CE-US was obtained on the six-month follow-up from patients included in the CE-US study. Six patients underwent a second examination with CE-US at 12 months. The results were included in the study as a second analysis.
All US examinations were performed using a high-end US system (Aplio XG, Toshiba, Otawa, Japan) in combination with an abdominal transducer.

2.2.2.1. B-mode Ultrasound

Conventional US was used to identify the anastomosis with an appropriate frequency between 5 and 6 MHz according to the depth of the anastomosis. Detection of a thrombus was recorded in writing by the examiner.

2.2.2.2. Color-coded Doppler ultrasound (CDUS)

CDUS was performed after B-mode US and documentation of the findings in two planes (sagittal and transverse). The flow volume and peak velocity (Vpeak) were determined in the anastomosis and in the proximal and distal recipient vessel. This image plane was documented as a freeze frame and video clip (15 s). CDUS was performed at a frequency of 800 to 1200 Hz with adjustment of the CDUS gain to ensure artifact-free documentation of the findings.

2.2.2.3. Contrast-enhanced Ultrasound (CE-US)

CE-US was performed using a dedicated US technique for contrast-enhanced imaging with a low mechanical index (MI<0.1) and low tissue signal (APO 0.75 %). This technique allows the investigator to follow the signal from the contrast agent microbubbles over time. The contrast agent used was SonoVue^{TM} (Bracco Altana Pharma GmbH, Germany) and US was performed after administration of bolus.

First bolus administration: The intravenous (IV) contrast bolus consisted of 1.2 ml of US contrast agent in combination with 5 ml of saline solution and was

injected into the left cubital vein over 4 to 5 sec (standardized procedure). The inflow phase was documented for 60 sec at a frame rate of 14 fps and stored digitally.

The postulated vortex formation at the anastomotic site was followed for 30 sec after a delay of 1 min 30 sec following contrast injection, with the use of the zoom function of the US system. The microbubbles were then destroyed using the flash technique and renewed influx was followed.

Second bolus administration: The second bolus of 1.2 ml was administered after a delay of 10 min. Using so-called microflow imaging (MFI), multiple consecutive frames were summed over time to form a single image. A video sequence (30 sec) and freeze frames served to document wall deposits (thrombus, intimal hyperplasia) in the anastomotic area. MFI [64] was also performed with a low mechanical index (MI<0.1).

Vortex evaluation: The evaluation of the vortex was primarily based on its subjective visualization by the two investigators with the help of the techniques described above . To achieve further objectivity, the course of a single microbubble was traced using a commercially not available software (Bubblio, Medical Engineering Laboratory, Toshiba Corporation, Tochigi, Japan) produced by Dr. Kamiyama for the needs of this study. This software was able to trace the vortical course of a bubble, provided that it remained on the scan plane. The effectiveness of this software has not been previously validated.

Ultrasound protocol:	5 MHz transducer
	0.754 % acoustic power
	≤ 0.1 mechanical index
	Contrast harmonic imaging (CHI)
	Microflow imaging (MFI)
	Frame rate of 14 fps
	2 times 1.2 ml SonoVue as IV bolus + 0.5 ml saline

2.2.2.4. Digital subtraction angiography (DSA)

Serial angiograms of the operated leg were obtained following fine-needle puncture of the ipsilateral femoral artery and automatic injection of about 100 ml of contrast agent (Imeron 300®, Altana Pharma, Konstanz, Germany). Pelvic-leg angiography was performed in selected patients when clinical indication was present.

2.3. Data analysis and statistics

2.3.1. Clinical series study

Collected epidemiologic data included age, gender, coronary artery disease, diabetes mellitus, hypertension, nicotine dependence, hyperlipidemia, renal insufficiency, creatinine values, Fontaine stage, number of patent tibial/peroneal vessels, prior operations, current medication such as statins or β-blockers, prior PTA of the ipsilateral SFA. Patients' data were collected in an SPSS 14.00 (SPSS Inc, Chicago, USA) database and Kaplan Meier curves were created with Stata 10.0 (StataCorp, Texas, USA).

2.3.2. Contrast-enhanced ultrasound study

Visualization of the distal bypass anastomosis was evaluated with CDUS and CE-US.

Hemodynamic features were assessed for both CDUS and CE-US using the representative freeze frames and the documented video clips. Assessment was done on a 3-point analogue scale (1, feature present; 2, feature not present; 3, unclear).

Immediately after each examination, the principal investigator (referred to as investigator 1) assessed the data for the presence of vortex formation, MIH, and the presence of thrombus using the 3-point scale. A second investigator (referred to as investigator 2), who reviewed the video of the principal investigator's examination and was blinded to the clinical findings, then repeated the analysis of the US examination performed by investigator 1.

If US demonstrated abnormal wall deposits, conventional angiography was performed within two weeks.

3.0. Results

3.1. Clinical Study

3.1.1. Patients' demographics

Between March 2005 and August 2007, 135 above-knee femoropopliteal bypasses were implanted in 134 patients using the Dynaflo® graft (Bard Peripheral Vascular Inc., Tempe, AZ) in 3 certified vascular centres in Germany. Table 3 summarizes the demographics and cardiovascular risk factors of the group at the point of the enrolment in the study, typical for patients with PAD. The majority of patients was male (n=110) and the overall mean age was 66 years (range 39-86). 112 patients (83 %) were prior to surgery on antiplatelet therapy with aspirin, 60% were under ACE-inhibitors and 58% under β-blockers. The mean preoperative creatinine value was 1.1mg/dl, range 0.5-2.7 (normal value <1.3mg/dl). In 39 cases (29%) the patients had undergone prior revascularization of the ipsilateral limb or iliac arteries as shown in Table 2. Six patients (4,6%) had undergone minor amputation of the affected limb prior to bypass implantation. The mean preoperative ABI of the group of patients with claudication was 0,6 (range 0,2-1,1). The patients with critical limb ischemia had a mean ABI of 0,3 (0,1-0,6). Five patients (5,1%) were excluded from the calculation of mean ABI because of an ABI over 1,5 and renal insufficiency or diabetes mellitus due to media sclerosis. The mean preoperative pain-free walking distance was in the group of patients with intermittent claudication 90 meters (range 10-200).

Table 3: Demographics and risk factors of 134 patients assigned to receive an above-knee Dynaflo® graft. One patient received a bilateral graft implantation

Risk factor		Grafts (n=135)	%°
Mean age		66 years	
Male / female		110 / 25	81/19
Stage of PAD*	Claudication	99	73
	Critical limb ischemia	36	27
Length of superficial femoral artery occlusion ≥ 20cm		125	92,6
Coronary artery disease		48	36
Prior myocardial infarction		30	22
Diabetes mellitus		42	31
NIDDM†		23	17
IDDM‡		19	14
Hypertension		113	84
Hyperlipidemia		56	42
Current and former smokers Current smokers		104 71	77 53
Renal insufficiency		31	23

° n=135 grafts (100%), *Peripheral arterial disease, † Non-insulin-dependent diabetes mellitus, ‡ insulin-dependent diabetes mellitus

The indication for revascularization was severe intermittent claudication in 99 (73%) and critical ischemia in 36 (27%) extremities. In more than 90% of the cases the occlusion of superficial femoral artery had a length of more than 20cm. In 39 cases (29%) the patients had undergone prior revascularization of the ipsilateral limb or iliac arteries as shown in Table 3. Two patients had undergone

prior femoropopliteal bypass implantation which was explanted and replaced by a Dynaflo® graft.

Table 4: Revascularization procedures of the affected limb preceding the above-knee bypass.

Interventions/ operations	N	%
None	96	71
PTA* of the superficial femoral artery (+/- stent)	10	7
PTA (+/- stent) of iliac arteries	15	11
Patch angioplasty of the femoral artery	7	5
Aortobifemoral bypass	7	5
Above-knee femoropopliteal bypass	2	1,5

*Percutaneous transluminal angioplasty

A patent below-knee popliteal artery was a requirement to perform above-knee revascularization. As demonstrated in Table 5, in 77 extremities (57%) all 3 crural vessels were patent, while 33 (24%) had 2 and 25 (19%) had only 1 patent tibial or peroneal vessel.

Table 5: Number of patent run-off vessels

Patent tibial / peroneal vessels	N	%
1	25	19
2	33	24
3	77	57

3.1.2. Procedure

Implantation of the pre-cuffed graft in the technique described above was the most common procedure performed.

In 12 patients (9%) an intraoperative optimization of inflow to the common femoral artery through balloon angioplasty or retrograde ring-assisted endarterectomy and angioplasty of the iliac arteries was necessary in addition to the femoropopliteal bypass. In one case thrombectomy and angioplasty of the outflow tibial vessels was performed simultaneously with the bypass implantation. No complications affecting the outcome of the femoropopliteal bypass resulted from the intervention on the iliac arteries. Of the 135 operated limbs, 31 underwent intraoperative angiography and the remaining 104 fine-needle angiography on postoperative day 3-5. There were no technical errors identified in the postoperative angiogram and therefore no early elective revisions were required.

Most of the grafts (88%) had a 7-mm diameter, and only 12% had an 8-mm diameter. The surgeon decided upon the diameter of the graft according to the diameter of the common femoral and the recipient artery. None of the grafts was ringed.

3.1.3. Postoperative anticoagulation

At the time of dismissal 81 patients (60%) were on anticoagulation with antiplatelet agents (aspirin or clopidogrel), 41 (30%) had a combination of aspirin and clopidogrel, and 12 (9%) were on oral warfarin. Two patients in our series had prior oral anticoagulation, so that a total of 10 patients (7,4%) had a change of anticoagulation to warfarin derivatives postoperatively. During follow-up the anticoagulation was changed from antiplatelet agents to warfarin derivatives in 11 patients. The indication was bypass occlusion with revision in order to preserve secondary patency in 5 cases (at 7, 8, 11, 13 and 17 months respectively) and thrombus in the distal anastomosis with high risk of distal embolization or occlusion in another one (at 12 months follow-up). The remaining

4 patients received warfarin derivatives for cardial indication between 6 and 18 months postoperatively.

3.1.4. Postoperative complications

Complications were observed in 39 patients (29%), with bypass failure (29 cases) and thrombus accumulation at the distal anastomosis being the most severe. No bypass infection was reported. All wound infections were successfully managed with repeated wound revisions and use of vacuum dressings in two cases. Postoperative haematoma had to be surgically evacuated in two cases, one of them combined with bypass revision due to occlusion after compression of the distal anastomosis. No perioperative cardiac infarctions, deep vein thrombosis or lung artery embolism occurred in this series.

3.1.5. Mortality

Six patients (4.4%) died during the follow-up period. One of them died within 30 days after operation corresponding to an operative mortality of 0.7%. The cause of death was a severe sepsis after gangrene of the lower extremity, which could not be salvaged despite successful revascularization, consequently requiring amputation above the knee. Of the remaining 5 patients, 4 died because of myocardial infarction and the other one of unknown cause.

3.1.6. Graft patency

After a mean follow-up of 18 months (range 1-31), 29 grafts had failed (occlusion or intervention required to preserve patency). The cumulative primary patency rate of the Dynaflo® graft at 6, 12, 18 and 24 months was 90%, 83%, 74% and 72.5%, respectively (Figure 5). The Kaplan-Meier analysis tables are available in the Appendix. Four patients underwent reoperation or change in anticoagulation therapy to preserve patency so that the primary assisted patency rates at 6, 12, 18 and 24 months were 92.4%, 86.2%, 77.6% and 74.6%, respectively (Figure 6). In a patient with a potentially failing graft, thrombus material was removed from the distal anastomosis by thrombectomy. Two patients underwent surgery

to optimize inflow and another patient was put on oral anticoagulation with warfarin because of thrombus material in the distal anastomosis. One of the two surgically treated patients had a severe stenosis of the common femoral artery above the proximal anastomosis, requiring endarterectomy and patch angioplasty extending to the proximal anastomosis. The second patient had a stenosis of the external iliac artery and the common femoral artery on 1-year follow-up treated with a hybrid procedure of balloon angioplasty and stent of the iliac artery and patch angioplasty of the common femoral artery. Secondary procedures included simple thrombectomy in 6 cases (4.4%), thrombectomy with revision of the distal anastomosis in another 6 patients (4.4%) and a redo procedure with below-knee revascularization in 3 patients (2.2%). The latter 3 patients with occluded Dynaflo® grafts were considered secondary failures and excluded from further evaluation in the study. Ten patients (7.4%) with bypass occlusion and stable claudication were managed conservatively. The resulting secondary patency rates at 6, 12, 18 and 24 months were 93%, 88.6%, 85.2% and 82,2%, respectively (Figure 7).

Figure 5: Kaplan-Meier analysis of primary patency

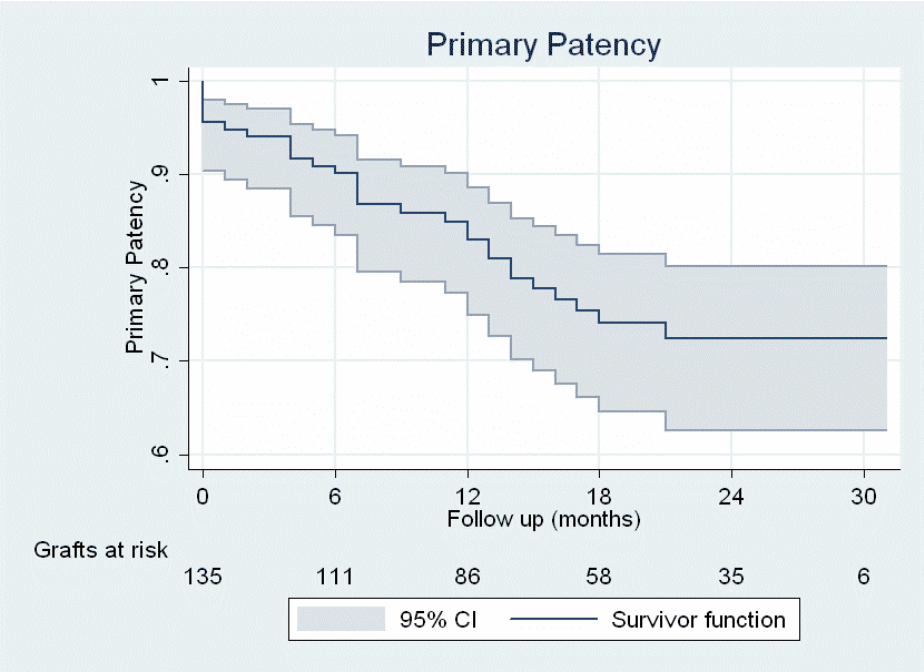


Figure 6: Kaplan-Meier analysis of primary assisted patency

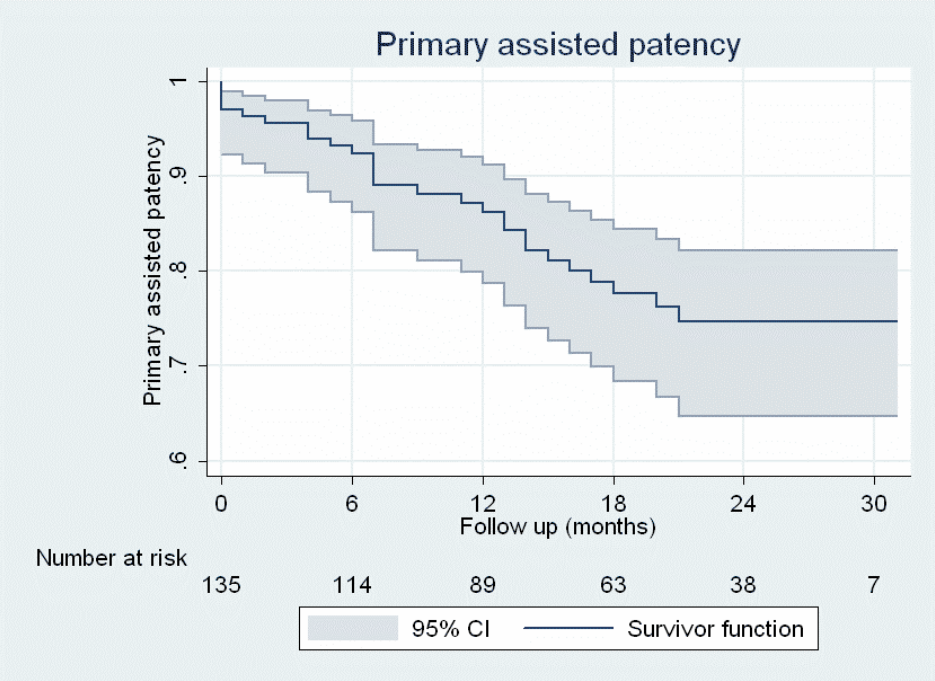
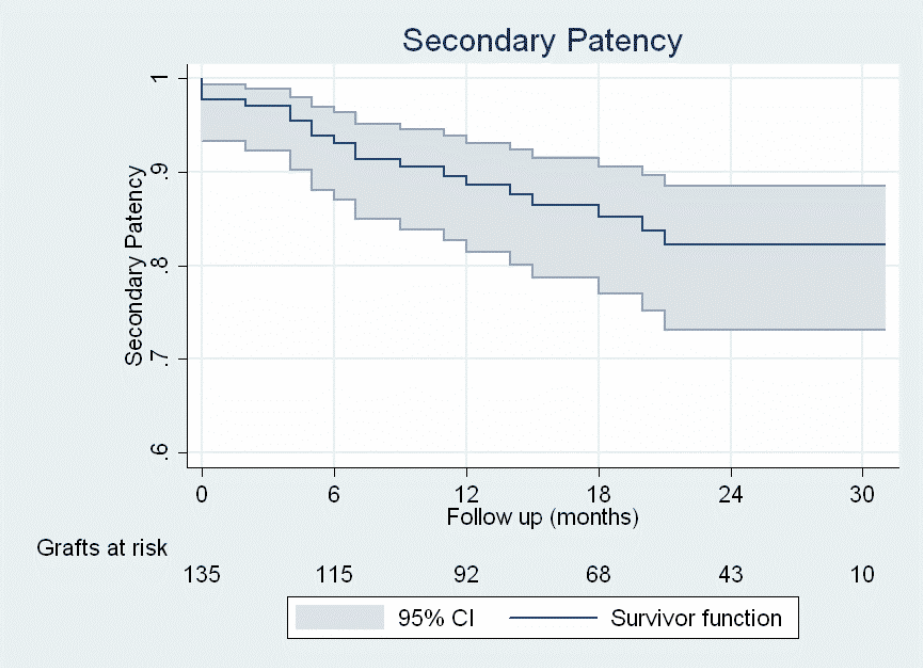


Figure 7: Kaplan-Meier analysis of secondary patency



3.1.7. Ankle-brachial index and walking distance

The mean postoperative ABI of the whole group was 1,2 (range 0,7 - 1,5). By claudicants the mean postoperative ABI was 1,2 (0,9 - 1,5), while patients with critical ischemia had a postoperative mean ABI of 1,0. (range 0,7 -1,2). The postoperative measurement on the treadmill was discontinued when the patient was free of pain after 200m. This occurred in 55% of the claudication patients postoperatively. The mean postoperative pain-free walking distance of the rest of the patients of the claudication group was 112m (range 50 - 200m).

3.1.7. Wound healing and limb salvage

Of the 36 patients with critical limb ischemia 25 had ulcers or gangrene and 11 had pain at rest. In the 11 cases of pain at rest, patients showed postoperative improvement of pain in all but one case. This patient had a vital extremity with good perfusion and the analgetic therapy was extended. The median visual analog scale for pain was preoperatively 7 and 1 week postoperatively 3.

Among the 25 extremities with ulcers or gangrene, complete healing of the wounds was achieved within 4 weeks postoperatively in 14 cases, while 9 patients required minor amputation. One patient required despite functioning bypass a below-knee amputation early postoperatively. No healing disorders of the amputation wounds were observed. The remaining patient had a chronic wound, which did not heal despite adequate revascularization and was further treated conservatively. Seven limbs (5%) were found unsalvageable and major amputation was performed despite a patent graft in 2 of these cases. The primary indication for revascularization of these limbs was critical limb ischemia in 5 cases and claudication in 2 cases. The cumulative 24-month limb salvage rate in the entire group of patients independent of the PAD stage was 95% and in the subgroup of 36 patients with critical limb ischemia 86%.

3.2. Results of contrast-enhanced ultrasound study

As stated above, all patients who underwent revascularization with the Dynaflo® graft in one of the three institutions (Chirurgische Klinik/Gefäßmedizin

Franziskus-Krankenhaus, Akademisches Lehrkrankenhaus der Charité – Universitätsmedizin Berlin) were offered the chance to participate in the CE-US study (Table 6).

Table 6: Epidemiologic data of the contrast-enhanced ultrasound study population

		N	%
Patients		31	
Extremities		32	100
Patients included in the CE-US imaging study		22	69
Reasons for exclusion n=10	Bypass occlusion	4	12.5
	No consent	4	12.5
	NYHA stage III-IV cardiac insufficiency	2	6
Fontaine stage	IIb	28	87.5
	III	2	6.3
	IV	2	6.3
TASC I classification	B	2	6.3
	C	13	40.6
	D	17	53.1
Coronary heart disease		5	15.6
Status post myocardial infarction		4	12.5
Diabetes mellitus		9	28.2
NIDDM		7	21.9
IDDM		2	6.3
Arterial hypertension		26	81.3
Hyperlipidemia		19	59.4
Smoking		14	43.8
Renal insufficiency		3	9.4

CE-US: contrast-enhanced ultrasound NYHA: New York Heart Association

SFA: superficial femoral artery (N)IDDM: (non-) insulin-dependent diabetes mellitus

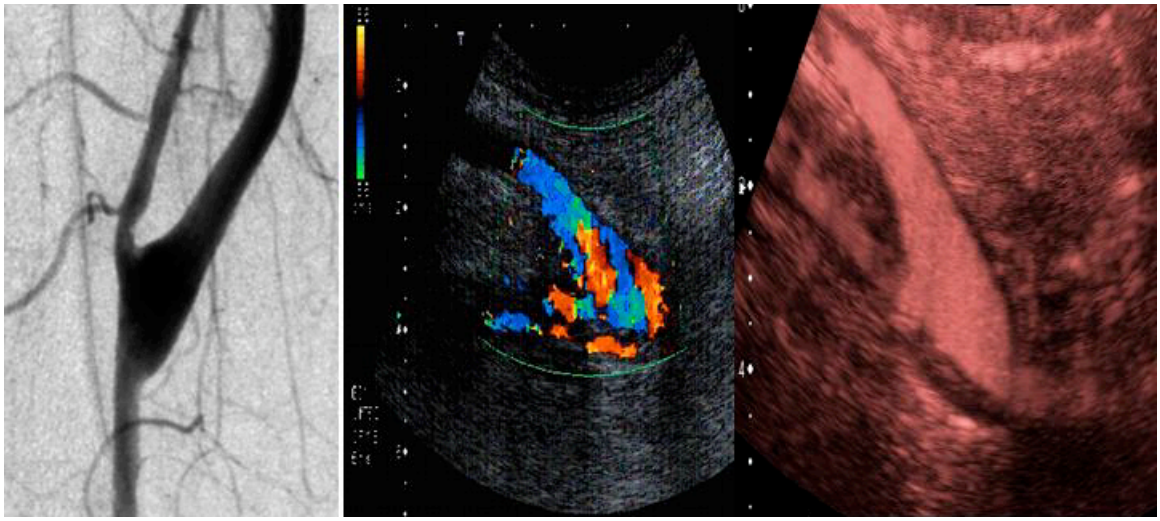
Twenty-two patients were included in the US study. All 22 extremities underwent hemodynamic evaluation by CDUS and CE-US (SonoVue^{TM}, Bracco) 6 months after implantation of the graft. Six patients underwent repeat CDUS and CE-US

12 months after surgery. Thus, a total of 28 examinations were available for analysis.

3.2.1. Visualization of the anastomosis and vortexing

Visualization of the shape of the anastomosis as a qualitative criterion of the suitability of the method for evaluation of the anastomotic site was assessed. Morphologic assessment of the anastomosis by CE-US was found to be nearly as good as with DSA (Figure 8).

Figure 8: Visualization of the prosthesis by conventional angiography (left), color-coded Doppler ultrasound (center), and contrast-enhanced ultrasound (right).



CDUS was less well suited for this purpose. Conventional CDUS enabled complete assessment of the distal bypass anastomosis in only 18 of 28 instances versus 26 of 28 instances using CE-US (Table 7). Only CE-US enabled reliable visualization of the anastomosis and recipient vessel in a single plane (22/28) compared with CDUS (7/28).

Table 7: Comparison of color-coded Doppler ultrasound (CDUS) and contrast-enhanced ultrasound (CE-US). Results of 28 examinations of above-knee Dynaflo® prosthesis bypass grafts.

Parameter		CDUS (%)	CE-US (%)
Visualization of the distal bypass anastomosis		18/28 (64)	26/28 (93)
Visualization of the anastomosis and recipient vessel in one plane		7/28 (25)	22/28 (79)
Demonstration of typical vortex formation at the anastomotic site	I1	11/28 (39)	21/28 (75)
	I2	6/28 (21)	20/28 (71)

I1: Investigator No. 1

I2: Investigator No. 2

CDUS was inadequate for assessing vortexing at anastomotic sites. CE-US demonstrated vortexing in 21 cases (75% of the prosthetic grafts, investigator 1) with investigator 2 identifying vortexing in 20 cases (71%). Using CDUS, investigator 1 identified vortexing in only 11 instances (39%), investigator 2 in only 6 instances (21%) (Figure 9, Table 7). This is the first time that the stable vortex formation is claimed to occur at the site of anastomosis and has also been confirmed in the clinical setting using CE-US. In all cases where the vortex was subjectively visible by both investigators, the Bubblio Software was able to trace an incomplete vortical movement of a single bubble, which was then lost from the scan plane. Only in a few cases was it possible to trace the complete vortical movement of one gas bubble (Figure 10).

Figure 9: Vortex formation was demonstrated by CE-US in a total of 21 instances (75% of the prosthetic grafts) by investigator 1 and in 20 instances by investigator 2 (71%). Using CDUS, investigator 1 identified vortexing in only 11 instances (39%), investigator 2 in only 6 instances (21%).

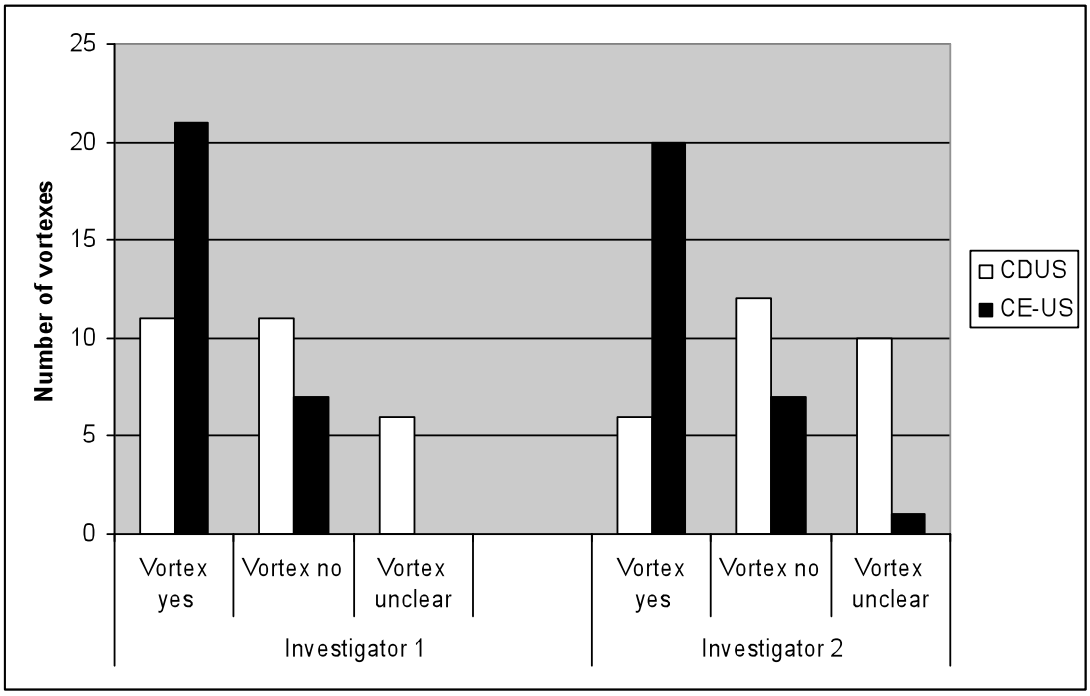
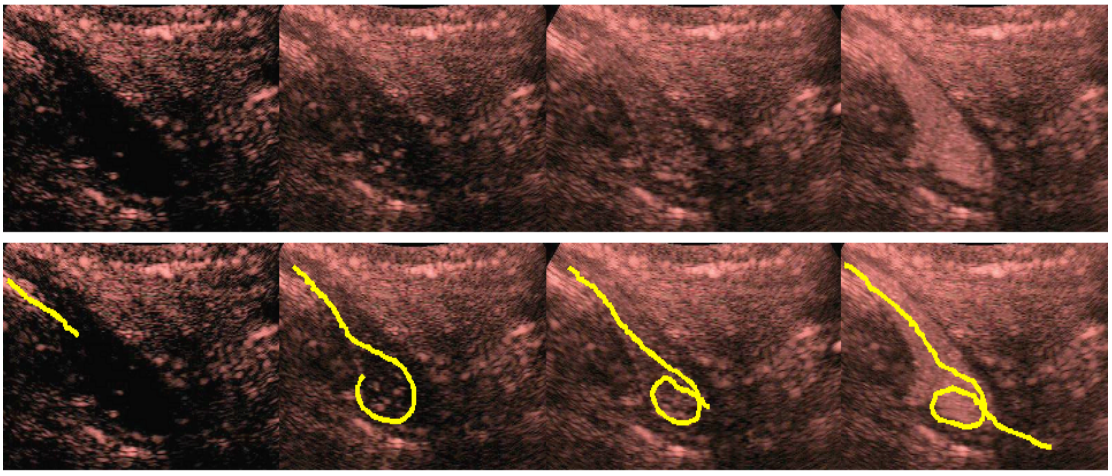


Figure 10: Appearance of a normal vortex in the distal anastomosis of the Dynaflo® prosthesis. The flow situation even in a deep anastomosis can be evaluated using contrast-enhanced ultrasound. Vortex formation is indicated by the yellow line (bright line in a black and white photo), which traces the motion of a gas bubble within the anastomosis.



3.2.2. Wall Deposits

CE-US was more accurate in detecting thrombotic wall deposits or MIH (investigator 1: 12/28, 42% / investigator 2: 11/28, 39%) compared with CDUS (investigator 1: 2/28, 7% / investigator 2: 2/28, 7%).

In 8 of 12 grafts, in which thrombus or IH were identified through CE-US the wall deposits were marginal (<5mm) and were not considered of clinical relevance. In the remaining 4 cases, DSA was performed confirming the findings of the CE-US in comparison to the postoperative DSA. In two cases therapeutic consequences were drawn (change of anticoagulation and thrombectomy) and in the other 2 cases the wall deposit was considered not significant and no further intervention was performed.

Estimating flow volume and peak velocity (V_{peak}) in the anastomosis and in the proximal and distal recipient vessel did not contribute to an improved description of the anastomotic situation. In particular, there were no differences in terms of vortexing and presence of thrombotic wall deposits.

4. Discussion

The patency of peripheral bypasses and especially of femoro-popliteal bypasses is influenced by several factors. They can be generally divided into patient- and bypass-related factors.

Patient-related factors include age, gender, smoking, hyperlipidemia, diabetes mellitus and hypertension as well as hypercoagulative states as independent risk factors[65,66]. An adequate management of these risk factors is essential before proceeding to surgical therapy and also thereafter. The number of patent below-knee arteries as well as the stage of PAD have also been described to affect bypass patency[66,67].

Bypass-related factors which affect bypass patency include the type of conduit used, the diameter of the graft and the surgical technique[68]. Several studies have proven that large-calibre grafts (>7mm) achieve better patency results[46,67,69].

Surgical technique remains a corner stone of any revascularization with decisive influence on outcome.

The issue regarding the best bypass material was addressed by several published meta-analyses which proved the superiority of vein not only for femoro-distal but also for above-knee bypasses[38,39,70]. Nevertheless many vascular surgeons are reluctant to sacrifice an available greater saphenous vein for a revascularization above the knee, because it could be required for a more critical below-knee revascularization in the future.

The ideal prosthetic vascular bypass graft should replicate the mechanical properties of native artery perfectly to maximize patency. In particular, it would demonstrate viscoelasticity for efficient pulsatile flow, matched compliance with the recipient artery to prevent MIH and have a burst pressure well above the physiological range of hemodynamic pressures. Moreover, this ideal prosthetic graft should have a near physiological inner surface similar to that of the endothelium[71].

In a systematic analysis of all different hemodynamic and mechanical values of a vein graft in the arterial system, Dobrin et al.[72] demonstrated that low flow

velocity is associated with MIH. The low shear-stress hypothesis states that low flow velocity results in longer contact of blood elements with the wall of the vessel and consequently elevated risk of MIH development[58,59,73].

This theory resulted in suggesting that optimization of the geometry of the distal anastomosis could prevent stagnation points and reduce wall shear stress at the critical locations of the anastomosis[50,74]. One possible solution was the development of the so-called pre-cuffed grafts to achieve a stable vortex over diastole and systole.

The hypothesis behind the pre-cuffed graft is that of the suppression of MIH through optimization of hemodynamic forces in the distal anastomosis and is based upon evidence of an inverse relationship between mean wall shear stress and MIH[61,62,74].

As a result, an ePTFE graft became commercially available in 1999 (Distaflo®, Bard Peripheral Vascular Inc., Tempe, AZ) and another one (FCPP)[49] was experimentally tested in a clinical trial, both promising better patency rates through optimized hemodynamic patterns in the distal anastomosis.

Two clinical trials[50,63] demonstrated non-inferiority concerning primary, secondary graft patency as well as limb salvage rates with use of a pre-cuffed ePTFE graft (Distaflo®, Bard Peripheral Vascular Inc., Tempe, AZ) as compared to a vein-cuffed ePTFE graft for infragenicular arterial bypasses.

4.1. Interpretation of clinical study results

In the era of endovascular surgery with the application of innovative techniques such as subintimal recanalization, laser assisted techniques and flexible or covered stents in the area of the superficial femoral artery, conventional vascular surgery appears to be on retreat[24,26-30]. Nevertheless, although the idea of achieving endovascular recanalization of the femoral artery also in TASC C/D lesions and reserving surgery for cases of failed endovascular therapy is appealing, no controlled randomized studies exist to compare bypass with PTA and give a reliable answer. To date long-term results of PTA are still inferior to surgery[2,75]. Therefore femoro-popliteal bypass remains the gold standard for

chronic occlusions of the superficial femoral artery longer than 20cm in most vascular centres.

The 5-year patency rate of ePTFE above-knee femoropopliteal bypass is estimated between 35%-52% in randomized trials with vein achieving cumulative patency of 74%-76%[43,52,70,75,76]. The manufacture of the Dynaflo® graft (Bard Peripheral Vascular Inc., Tempe, AZ) aimed to improve patency rates of above-knee femoropopliteal bypasses by optimizing the hemodynamic patterns through anastomotic engineering of the distal anastomosis.

In the present prospective multicenter study the cumulative primary and secondary patency on 24 months follow-up were 72,5% (CI 62.6%-80.2%) and 82% (CI 73.1%-88.5%) respectively. The cohort consisted of patients with intermittent claudication in 73% of the cases, which also explains the low rate of major amputation (5%) in our series. However, two limb losses occurred in patients who suffered from intermittent claudication before the primary operation. Both patients presented on follow-up with progressed infra-popliteal atherosclerotic disease and bypass occlusion. In one patient embolization of the tibial vessels from a thrombus at the cuff of the graft was suspected.

In the present series four patients were diagnosed with relevant thrombus formation in the cuff of the distal anastomosis. In two patients the thrombus mass was not considered clinically relevant and required no intervention, one patient was managed with oral anticoagulation and the last patient underwent successful bypass revision to remove the thrombus from the distal anastomosis.

Formation of thrombus within the cuff of the distal anastomosis could be a major drawback of this graft. Furthermore, in our experience surgical thrombectomy of an occluded graft frequently required revision of the distal anastomosis to fully extract the thrombus mass from the cuff, which was difficult to achieve over an inguinal approach with “Fogarty maneuver” alone. For the same reason an intra-operative DSA is considered obligatory in all cases of Dynaflo® graft revision.

The Dynaflo® graft is made at the cuff area of thinner ePTFE, which could provide better viscoelastic properties and better compliance. The cuff of the graft

requires therefore especially gentle handling during suture of the distal anastomosis.

Major drawbacks of our study are its descriptive character and the lack of a comparative analysis of a control group of patients undergoing implantation of another graft. However, data from other studies as well as meta-analyses are available and can be used for cautious comparison to the results of the present study.

In several studies ePTFE achieved cumulative patency rates of 50%-77% [41,44,52,77] after 2 years follow-up.

Klinkert et al. reported in their meta-analysis cumulative primary patency of 67% for ePTFE grafts at 2-year follow-up when all studies were reviewed (2520 grafts) and 69% when only controlled randomized trials were included in the analysis (643 grafts)[38].

Therefore, considering the limitations of this study, it could be cautiously concluded that with 72,5% primary and 82% secondary cumulative patency the Dynaflo® graft seems to perform at least equally well at two years follow-up compared with normal ePTFE grafts.

Only randomized controlled studies and meta-analyses could give a definite answer to the question of which is the best conduit for the above-knee femoro-popliteal bypass at an adequate level of evidence.

4.2. Interpretation of contrast-enhanced ultrasound study results

Various imaging modalities can theoretically be used for in vivo hemodynamic assessment of anastomotic sites of bypass grafts. However, such an imaging modality should have a fairly high resolution and provide for dynamic analysis of flow. A so-called cine loop technique on the basis of conventional DSA has been proposed[78]. Other candidates are the cross-sectional imaging modalities. US is the most popular modality, mainly because it is inexpensive and readily available. Blood flow has traditionally been assessed by CDUS; improved hemodynamic assessment has become possible with the advent of new US contrast agents and technical advances[64,79-82].

The study presented here enabled for the first time evaluation of the in vivo hemodynamic situation at the distal anastomosis of the Dynaflo® bypass graft using CE-US. The vortex formation, so far only demonstrated in vitro, is supposed to be relatively stable over the cardiac cycle, minimizing this way flow reduction and flow stagnation near the wall at anastomotic sites[74,83].

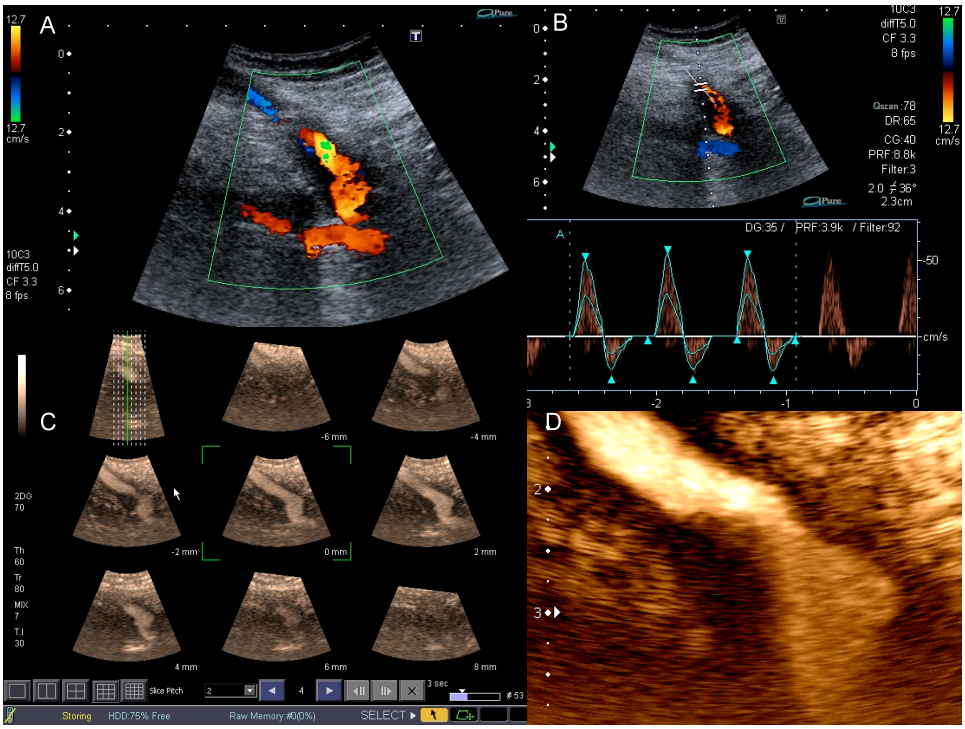
In our patient population, this type of vortex formation was demonstrated by CE-US in 21 cases, while CDUS revealed only 11 instances. We confirmed for the first time the presence of vortex formation at the anastomotic site, so far only demonstrated in vitro. This was possible because of the CE-US technique's ability to demonstrate the course of only a few microbubbles in the vascular lumen. The microbubbles were found to follow a circular path, indicating vortexing. CDUS is technically inadequate to demonstrate this kind of motion of flowing blood.

Moreover, conventional CDUS enabled adequate evaluation of the distal bypass anastomosis in only 18 of 28 examinations, while CE-US enabled complete evaluation in 26 of 28 instances. Only CE-US allowed reliable visualization of the anastomosis and recipient vessel in one plane compared with CDUS (22/28 versus 7/28).

CE-US improved visualization not only of the anastomosis, including the proximal and distal portions of the recipient artery, but also of the vessel wall.

CE-US enables one-stop shop diagnostic evaluation of the hemodynamic situation and demonstration of thrombi or MIH. CE-US was more accurate in detecting thrombotic wall deposits or MIH (Figure 11).

Figure 11: Appearance of the anastomosis 6 months after surgery. CDUS (A) reveals slightly turbulent flow and possible wall deposits. Pulsed-wave Doppler demonstrates biphasic spectra and no significant flow acceleration (B). CE-US (C) enables good evaluation of the entire anastomosis with clear delineation of the mural thrombus/intimal hyperplasia (D).



It is expected that three-dimensional CE-US will further improve the spatial and temporal quantification of flow phenomena at anastomotic sites as suggested by results reported by How et al.[84]. The mere fact that most models assume planar anastomosis while this is not so in vivo in most cases has consequences for the hemodynamic situation at anastomotic sites.[84]

Blood flow at the site of an end-to-side anastomosis depends not only on the type of anastomosis but also on a number of other factors such as height and length of the cuff, angle of the bypass prosthesis relative to the recipient artery, and resistance in the outflow tract[73]. A numeric simulation that took into account not only wall shear stress but also the interactions of blood components with the vessel wall has clearly shown that anastomotic engineering is only one

of a number of factors that affect the patency rate of alloplastic prosthetic bypass grafts[85].

Using CE-US, we were not able to show altered hemodynamics with loss of the typical vortex formation when wall deposits were present, because statistical analysis was not feasible due to the high number of marginal wall deposits (8 of 12 cases). However, it was not an endpoint of the study to correlate the results of CE-US with the patients' clinical course. However, further clinical studies are required in order to prove whether the characteristic hemodynamic feature of the Dynaflo® anastomosis is of any clinical relevance.

5. Conclusions

In a prospective multicenter cohort study the Dynaflo® graft achieved satisfactory patency- and limb-salvage rates with low perioperative mortality.

I. At 6, 12, 18 and 24 months primary patency was 90%, 83% 74% and 72,5%, respectively.
II. Primary assisted patency rates at 6, 12, 18 and 24 months were 92.4%, 86.2%, 77.6% and 74.6%, respectively.
III. The secondary patency rates were 93%, 88.6% 85.2% and 82.2%, respectively.
IV. The cumulative 24-month limb salvage rate in the entire group of patients independent of the PAD stage was 95% and in the subgroup of 36 patients with critical limb ischemia 86%.
V. Overall mortality after 18-month median follow-up was 4,4% and perioperative (30-day after operation) mortality was 0,7%.

The contrast-enhanced color-coded Doppler sonography (CE-US) study of the Dynaflo® graft was able to demonstrate the following points:

I. CE-US enabled in-vivo evaluation of hemodynamic patterns at the distal anastomosis of pre-cuffed grafts with demonstration of stable vortices for the first time**.**
II. CE-US was more accurate in detecting thrombotic wall deposits or MIH.

6. Abbreviations

PAD: peripheral arterial disease

US: ultrasound

CDUS: conventional color-coded Doppler sonography

CE-US: contrast-enhanced ultrasound

MIH: myointimal hyperplasia

PTFE: polytetrafluoroethylene

ePTFE: expanded polytertafluoroethylene

ABI: ankle-brachial index

DSA: digital subtraction angiography

CI: confidence interval

CTA: computed tomography angiography

MRA: magnetic resonance angiography

RSFAE: remote superficial femoral artery endarterectomy

iv: intravenous

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8. Appendix

Appendix Table 1: Kaplan-Meier analysis table for primary patency

Time, months	Total, No	Failed, No	Lost, No	Function	Error	95% CI
.01	135	6	0	0.9556	0.0177	0.9038-0.9798
1	129	1	0	0.9481	0.0191	0.8943-0.9749
2	128	1	4	0.9407	0.0203	0.8850-0.9699
3	123	0	3	0.9407	0.0203	0.8850-0.9699
4	120	3	4	0.9172	0.0239	0.8554-0.9533
5	113	1	1	0.9091	0.0251	0.8454-0.9474
6	111	1	1	0.9009	0.0261	0.8353-0.9413
7	109	4	4	0.8679	0.0299	0.7958-0.9158
8	101	0	4	0.8679	0.0299	0.7958-0.9158
9	97	1	2	0.8589	0.0309	0.7851-0.9088
10	94	0	4	0.8589	0.0309	0.7851-0.9088
11	90	1	3	0.8494	0.0320	0.7735-0.9014
12	86	2	1	0.8296	0.0342	0.7498-0.8859
13	83	2	3	0.8096	0.0362	0.7263-0.8698
14	78	2	4	0.7889	0.0381	0.7022-0.8529
15	72	1	4	0.7779	0.0391	0.6894-0.8440
16	67	1	3	0.7663	0.0402	0.6758-0.8346
17	63	1	4	0.7541	0.0414	0.6616-0.8247
18	58	1	6	0.7411	0.0427	0.6462-0.8142
19	51	0	1	0.7411	0.0427	0.6462-0.8142
20	50	0	5	0.7411	0.0427	0.6462-0.8142
21	45	1	3	0.7247	0.0448	0.6256-0.8016
22	41	0	3	0.7247	0.0448	0.6256-0.8016
23	38	0	3	0.7247	0.0448	0.6256-0.8016
24	35	0	3	0.7247	0.0448	0.6256-0.8016
25	32	0	8	0.7247	0.0448	0.6256-0.8016
26	24	0	2	0.7247	0.0448	0.6256-0.8016
27	22	0	5	0.7247	0.0448	0.6256-0.8016
28	17	0	6	0.7247	0.0448	0.6256-0.8016
29	11	0	5	0.7247	0.0448	0.6256-0.8016
30	6	0	5	0.7247	0.0448	0.6256-0.8016
31	1	0	1	0.7247	0.0448	0.6256-0.8016

Appendix Table 2: Kaplan-Meier analysis table for primary assisted patency

Time, months	Total, No	Failed, No	Lost, No	Function	Error	95% CI
.01	135	4	0	0.9704	0.0146	0.9230-0.9888
1	131	1	0	0.9630	0.0163	0.9133-0.9844
2	130	1	4	0.9556	0.0177	0.9038-0.9798
3	125	0	3	0.9556	0.0177	0.9038-0.9798
4	122	2	4	0.9399	0.0206	0.8834-0.9695
5	116	1	1	0.9318	0.0220	0.8729-0.9639
6	114	1	1	0.9236	0.0233	0.8626-0.9582
7	112	4	4	0.8906	0.0277	0.8221-0.9338
8	104	0	4	0.8906	0.0277	0.8221-0.9338
9	100	1	2	0.8817	0.0288	0.8112-0.9271
10	97	0	4	0.8817	0.0288	0.8112-0.9271
11	93	1	3	0.8722	0.0300	0.7995-0.9199
12	89	1	1	0.8624	0.0312	0.7874-0.9124
13	87	2	2	0.8426	0.0335	0.7634-0.8970
14	83	2	4	0.8223	0.0356	0.7393-0.8810
15	77	1	4	0.8116	0.0367	0.7267-0.8725
16	72	1	3	0.8004	0.0379	0.7133-0.8635
17	68	1	4	0.7886	0.0391	0.6992-0.8541
18	63	1	6	0.7761	0.0405	0.6843-0.8442
19	56	0	1	0.7761	0.0405	0.6843-0.8442
20	55	1	5	0.7620	0.0421	0.6670-0.8332
21	49	1	3	0.7464	0.0440	0.6477-0.8212
22	45	0	4	0.7464	0.0440	0.6477-0.8212
23	41	0	3	0.7464	0.0440	0.6477-0.8212
24	38	0	3	0.7464	0.0440	0.6477-0.8212
25	35	0	8	0.7464	0.0440	0.6477-0.8212
26	27	0	2	0.7464	0.0440	0.6477-0.8212
27	25	0	6	0.7464	0.0440	0.6477-0.8212
28	19	0	7	0.7464	0.0440	0.6477-0.8212
29	12	0	5	0.7464	0.0440	0.6477-0.8212
30	7	0	6	0.7464	0.0440	0.6477-0.8212
31	1	0	1	0.7464	0.0440	0.6477-0.8212

Appendix Table 3: Kaplan-Meier analysis table for secondary patency

Time, months	Total, No	Failed, No	Lost, No	Function	Error	95% CI
.01	135	3	0	0.9778	0.0127	0.9327-0.9928
2	132	1	4	0.9704	0.0146	0.9230-0.9888
3	127	0	3	0.9704	0.0146	0.9230-0.9888
4	124	2	4	0.9547	0.0181	0.9019-0.9794
5	118	2	1	0.9385	0.0211	0.8808-0.9688
6	115	1	1	0.9304	0.0224	0.8703-0.9632
7	113	2	4	0.9139	0.0249	0.8498-0.9514
8	107	0	4	0.9139	0.0249	0.8498-0.9514
9	103	1	2	0.9050	0.0262	0.8386-0.9450
10	100	0	4	0.9050	0.0262	0.8386-0.9450
11	96	1	3	0.8956	0.0275	0.8266-0.9382
12	92	1	1	0.8859	0.0289	0.8143-0.9310
13	90	0	5	0.8859	0.0289	0.8143-0.9310
14	85	1	4	0.8755	0.0304	0.8011-0.9233
15	80	1	4	0.8645	0.0319	0.7872-0.9152
16	75	0	3	0.8645	0.0319	0.7872-0.9152
17	72	0	4	0.8645	0.0319	0.7872-.09152
18	68	1	6	0.8518	0.0339	0.7704-0.9060
19	61	0	1	0.8518	0.0339	0.7704-0.9060
20	60	1	5	0.8376	0.0362	0.7515-0.8959
21	54	1	3	0.8221	0.0387	0.7307-0.8848
22	50	0	4	0.8221	0.0387	0.7307-0.8848
23	46	0	3	0.8221	0.0387	0.7307-0.8848
24	43	0	3	0.8221	0.0387	0.7307-0.8848
25	40	0	9	0.8221	0.0387	0.7307-0.8848
26	31	0	2	0.8221	0.0387	0.7307-0.8848
27	29	0	7	0.8221	0.0387	0.7307-0.8848
28	22	0	7	0.8221	0.0387	0.7307-0.8848
29	15	0	5	0.8221	0.0387	0.7307-0.8848
30	10	0	9	0.8221	0.0387	0.7307-0.8848
31	1	0	1	0.8221	0.0387	0.7307-0.8848

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Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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Erklärung

„Ich, Nikolaos Tsilimparis, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: ***„Anastomotic engineering in above-knee femoropopliteal bypass with pre-cuffed distal end. Clinical results of a multi-centre cohort study and investigation of in vivo hemodynamics.“*** selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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