THE FATE OF REVASCULARIZATION FOR PERIPHERAL ARTERIAL DISEASE

EXPERIMENTAL AND CLINICAL STUDIES

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE

Traditionally, peripheral arterial disease (PAD) encompasses atherosclerosis of non-coronary as well as non-cerebral arteries, including those of the limbs, aorta, carotids, and visceral arteries. Most typically, PAD refers to atherosclerotic occlusive disease involving the arteries serving the lower limb, which accounts for the majority of cases. Recent data show that over 200 million people worldwide have PAD, with an estimated 12 to 20% of people older than 65 years affected in the United States alone.[1,2] Due to a combination of increased life expectancy as well as dietary and lifestyle habits, prevalence of PAD increased by 23.5% globally over a decade and this number is expected to rise even further.[1] Occlusive arterial disease of the lower extremities is often considered a marker of extensive systemic atherosclerotic burden, with patients frequently suffering from multiple other co-existing medical conditions, including coronary artery disease, congestive heart failure, and chronic kidney disease. As a result, PAD continues to pose a major challenge for healthcare and western society.

Atherosclerosis of the peripheral arteries is characterized by a chronic and slowly developing process causing narrowing and/or occlusion of the arteries. Atherosclerotic lesions are plaques composed of a central lipid core, connective tissue, inflammatory cells, and smooth muscle cells, all covered by a fibrous cap.[3,4] These plaques tend to localize at the bifurcations or proximal segments of the arteries, with the femoropopliteal segment affected most frequently. While many patients will remain asymptomatic throughout their life, others may develop symptoms in different stages of severity mostly depending on the degree of stenosis at the vascular site. Intermittent claudication consists of pain or cramps distal to the diseased arterial segment, triggered by a reproducible distance of walking and relieved quickly and consistently by rest. In more advanced stages, patients may experience pain in rest or suffer from tissue loss (non-healing ulcers or gangrene), an indication of chronic limb-threatening ischemia (CLTI) and reflective of a state of resting blood flow that does not meet the requirements of basic metabolic rates of these tissues. This is usually caused by diffuse multilevel arterial lesions in the limb.

Although two different disease entities, both claudication and CLTI are associated with adverse clinical and surgical outcomes, including mortality, cardiovascular morbidity, and adverse limb events.[5-10] The progression of disease in CLTI, however, is excessively worse than that of claudication, with one-year amputation rates of approximately 30% irrespective of treatment and one-year mortality as high as 25% after diagnosis.[11] The primary aims of treatment in patients with atherosclerosis of the lower extremities are limb salvage, symptom relieve, and improvement of quality of life. Due to the systemic nature of atherosclerotic disease, management of these patients also aims to lower the risk of major cardiovascular events, such as myocardial infarction, stroke, and ultimately prolong survival. Aggressive modification of known risk factors, so-called secondary prevention, is recommended for all patients with PAD and entails intensive lifestyle interventions, including smoking cessation, weigh reduction, and optimal medical management. As an

alternative to surgical interventions, the primary mode of treatment for most PAD patients is some kind of supervised exercise training, which has been shown to reduce symptoms and improve functional capacity, such as walking distance and pain-free walking time.[12,13] Successes notwithstanding, selected patients with impairment of daily activities due to claudication and those with CLTI, need some form of limb revascularization providing durable relief of symptoms. Alleviation of symptoms and restoration of blood flow to the distal parts of the limb can be attempted either by surgical revascularization, consisting primarily of a bypass procedure or endarterectomy, and catheter-based endovascular interventions, also known as balloon angioplasty with or without stent. With bypass an alternative conduit for blood flow is created to circumvent the stenotic lesions, while endovascular intervention is accomplished by using a balloon-tipped catheter usually inserted through the femoral artery in the groin to help widen the stenotic lesion in the artery, which is often combined with permanent placement of a stent.

Despite the enormity of the population at risk and a paradigm shift from open bypass to less invasive endovascular procedures[14-16], current vascular practice suffers from lack of consensus in regard to treatment of PAD. Unlike other subspecialties, like coronary or carotid surgery, there are few high-quality randomized, controlled trials that provide level I evidence to support either one strategy over the other.[17,18] As a result, practice patterns may vary by country, hospital, and even by physicians within the same institution. This is further illustrated by the absence of clear guidelines and recommendations from major societies, which impedes publication of new guidelines.[11,19-21] Overall, endovascular intervention has been associated with lower periprocedural morbidity, whereas others have suggested superior long-term clinical durability after bypass.[17,22-26] Aside from the intervention performed, outcomes of revascularization depend on the extent of the lesions, in particular the condition of the inflow and outflow arteries, and the length and number of stenoses and/or occlusions of the diseased segment, in combination with clinical factors and comorbid conditions.[11] Despite many technical advances in both treatment modalities, such as drug-coated balloons and drug-eluting stents or grafts, patency and limb salvage rates only improved marginally.[27,28]

Without exception, the major drawback for all interventions treating atherosclerotic occlusive disease remains durability. Recurrent narrowing of the intervened segment of the artery (restenosis) is an important clinical complication limiting patency rates after revascularization. Restenosis is the result of a complex pathologic response of vascular remodeling that triggers intimal hyperplasia and can occur after both angioplasty or bypass over a time frame of months to years. Delayed one-year failure rates of bypass procedures occur in approximately 30-35%, with up to 70% of patients requiring a reintervention to treat the restenotic lesion.[29] Prosthetic bypass grafts perform even worse and should not be used when an autologous graft is available. For endovascular interventions, long-term patency is even more of a concern with restenosis rates of greater than 40-50% within one-year, although vascular stents reduced these rates notably.[24,30-33]

The first step in improving patency rates and limb preservation is understanding the mechanisms involved. Although the etiology remains an enigma, various proposed injury pathways may promote a pathologic cascade of reactions that result in vessel wall thickening. All vessel walls (artery and vein) consist of three layers. First, the intima, the inner lining of the vessel wall, that constitutes of an endothelium cell layer on top of the inner elastic lamina. Second, the medial layer composed of smooth muscle cells and extracellular matrix supported by the outer elastic lamina. And finally, the adventitia, the outer layer of the vessel wall, which contains collagen, fibroblasts, and the vasa vasorum. Physical manipulation, exposure to increased hydrostatic forces, and shear stress-induced damage contribute to an injury response during and after the revascularization procedure and marks the starting point of vascular remodeling. While vascular remodeling is a normal physiologic process of structural adaptation, ongoing and uncontrolled remodeling may subsequently contribute to the pathophysiology of restenosis. Considerable damage to the endothelium exposes the subendothelial matrix and leads to platelet adhesion and aggregation. This predisposes a chronic inflammation reaction both in the intima and adventitia through recruitment and infiltration of leukocytes to the injury site. The last phase consists of smooth muscle cell differentiation, the hallmark of the restenotic process. In response to the abundance of intra- and extracellular stimuli, including growth factors, extracellular matrix proteins, cytokines, and cell-surface receptors, smooth muscle cells in the media modulate from a quiescent contractile to a synthetic motile state, which results in migration and proliferation to the intimal layer leading to arterial wall thickening.[32,34-37] Due to the unproportional smooth muscle cell and myofibroblast differentiation as well as progression of atherosclerosis, the functional diameter of the lumen becomes increasingly compromised. (Figure) Symptoms can reoccur when blood flow becomes hindered through restenosis and distal tissue ischemia advances.

Since recurrent symptoms and ischemia are strongly related to the progression and development of restenosis, it would appear that innovative and novel strategies to mitigate genomic alterations that result in intimal hyperplasia hold strong potential to improve outcomes after revascularization.[38-41] Firstly, the *ex vivo* time frame handling autologous or prosthetic grafts as well as the isolated, localized intervention of angioplasty offer a unique opportunity to use gene-based therapies with local drug delivery during the procedure and without unintended systematic consequences. Secondly, because of the wide array of genes involved in the complex pathophysiology of vascular remodeling, new targets can be modulated selectively, either by knockdown or stimulation, with an increasing inventory to mediate numerous of cellular pathways and molecular components.[42] Consequently, during the last two decades, there has been a widespread increase of interest by clinicians and researchers to develop devices and biomaterials that locally release drugs to prevent restenosis. Numerous therapeutic targets for restenosis have been identified; however, few have reached clinical studies and almost none made it into daily clinical practice.[29,41,43-46] The central theme will be targeted, local and sustained delivery of drugs and, although

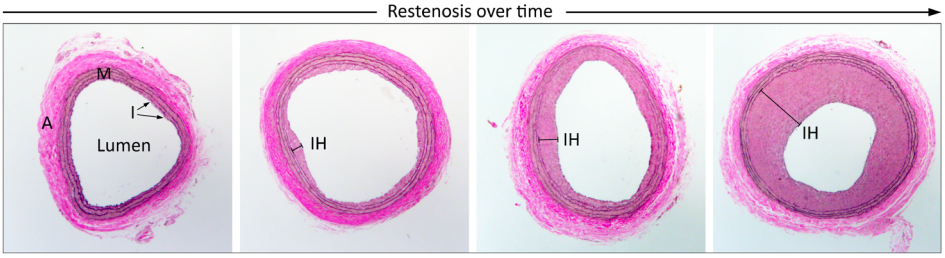

Figure. Progressive eccentric development of restenosis and lumen loss over time after revascularization. General histological features of arterial cross-sections following Verhoeff-van Gieson stain.
A: adventitia, *M*: media, *I*: intima, *IH*: intimal hyperplasia

great strides have been made, thus far no effective treatment has been developed to selectively target restenosis after revascularization. Increased understanding of cellular processes and gene functions implicated in intimal hyperplasia and restenosis, together with advances in gene delivery methods, offer incredible tools for molecular bioengineering of the vessel wall.

Objectives and outline of this thesis

This thesis sets out to assess the performance of lower extremity vascular interventions in occlusive arterial disease through large clinical data analysis and to investigate novel and innovative strategies that aims to mitigate restenosis and improve durability of revascularization.

Part I of this thesis is dedicated to determine current revascularization practices and assess risk factors for restenosis, adverse limb events, and mortality. **Chapter 2** investigates the optimal revascularization strategy for claudication and CLTI in patients without prior ipsilateral interventions and focuses on patient characteristics associated with selection of one strategy over the other. As diabetes poorly affects the prognosis of PAD, **Chapter 3** determines, in an institutional cohort, outcomes after vascular interventions, focusing on the severity of diabetes, in particular insulin-dependent and noninsulin-dependent diabetes. **Chapter 4** investigates new opportunities to optimize secondary preventive measures after vascular intervention and evaluates the role of renin-angiotensin system inhibitors. The importance of preoperative anemia, a more unconventional risk factor, on perioperative outcomes after bypass is discussed in **Chapter 5**, while long-term outcomes are examined in **Chapter 6**. Whether blood transfusions mitigate these anemia-associated risks is additionally evaluated in the two previous chapters. The purpose of **Chapter 7** is to assess the incidence of unplanned readmission after endovascular interventions and to identify patient- and procedure-specific risk factors, as well as in-hospital complications associated with readmissions. In case of a significant restenosis it is still common practice to

reintervene. In **Chapter 8**, the authors assess the impact of prior procedures on perioperative outcomes of a subsequent bypass in a large representative national registry from the United States.

In *Part II*, the aim is to develop efficient gene therapeutic strategies to intervene on the pathological process of restenosis after revascularization. These processes may include inhibition of smooth muscle cell differentiation and migration, improvement of endothelial function, and modulation of the inflammatory cascade that triggers intimal hyperplasia. In **Chapter 9** a new approach to inhibit the expression of one specific gene, trombospondin-2, is described and its effect on intimal hyperplasia in an animal model is further elucidated. **Chapter 10** corroborates these findings and investigates the concept of multiple gene knockdown in a preclinical animal model and evaluates the alterations in response to vascular injury

Finally, **Chapter 11** summarizes the findings of the previous chapters and discusses further perspectives of revascularization in patients with PAD.

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PART ONE

CLINICAL STUDIES

CHAPTER 2

PATIENT SELECTION AND PERIOPERATIVE OUTCOMES OF BYPASS AND ENDOVASCULAR INTERVENTION AS FIRST REVASCULARIZATION STRATEGY FOR INFRAINGUINAL ARTERIAL DISEASE

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ABSTRACT

Objective

The optimal initial revascularization strategy remains uncertain for patients with peripheral arterial disease (PAD). The purpose of this study was to evaluate current nationwide patient selection and perioperative outcomes of patients undergoing bypass or endovascular intervention for infrainguinal disease in those with no prior ipsilateral revascularization.

Methods

Patients undergoing non-emergent first-time infrainguinal revascularization were identified in the targeted vascular NSQIP 2011-2014 and stratified by symptom status (chronic limb-threatening ischemia [CLTI] or claudication). Patients treated with endovascular intervention were compared to those who underwent bypass. Multivariable logistic regression was used to evaluate current patient selection and to establish independent associations between first-time procedures and postoperative outcomes.

Results

Of 5,998 first-time infrainguinal revascularizations performed, 3,193 were bypass procedures (63% CLTI) and 2,805 endovascular interventions (64% CLTI). Current patient characteristics associated with an endovascular-first approach, as opposed to bypass-first, in CLTI patients were age ≥80, tissue loss, non-smoking, functional dependence, diabetes, dialysis, and infrapopliteal lesions, whereas age ≥80, non-white race, non-smoking, diabetes, and infrapopliteal lesions were associated with an endovascular approach for claudication. When comparing first-time endovascular intervention to bypass, there was no difference in 30-day mortality in CLTI (2.1% vs. 2.2%, OR 0.7 [0.4-1.1]) or claudication patients (0.3% vs. 0.6%). Among CLTI patients, endovascular-first was associated with lower rates of major adverse cardiovascular event (MACE) (3.6% vs. 4.7%, OR 0.6 [0.4-0.9]), surgical site infection (0.9% vs. 7.7%, OR 0.1 [0.1-0.2]) bleeding (8.5% vs. 17%, OR 0.4 [0.3-0.5]), unplanned reoperation (13% vs. 17%, OR 0.7 [0.5-0.8]), and unplanned readmission (17% vs. 18%, OR 0.8 [0.7-0.9]). Patients with claudication undergoing endovascular-first also had lower rates of MACE (0.8% vs. 1.6%, OR 0.4 [0.2-0.95]), surgical site infection (0.7% vs. 6.6%, OR 0.1 [0.04-0.2]), bleeding (2.3% vs. 6.0%, OR 0.3 [0.2-0.5]), unplanned reoperation (4.3% vs. 6.6%, OR 0.6 [0.4-0.9]), and unplanned readmission (5.9% vs. 9.0%, OR 0.6 [0.4-0.8]). Conversely, endovascular-first was associated with a higher rate of secondary revascularizations within 30 days for CLTI (4.3% vs. 3.1%, OR: 1.6 [1.04-2.3]) but not for claudication (2.6% vs. 1.9%, OR: 1.7 [0.9-3.4]).

Conclusions

An endovascular-first approach as a revascularization strategy for infrainguinal disease was associated with substantially lower early morbidity but not mortality, at the cost of higher rates of postoperative secondary revascularizations. As a national representation of first-time revascularizations, this study highlights the early endovascular perioperative benefit, although more robust long-term data are needed to adopt either one strategy over the other in select PAD patients.

INTRODUCTION

The admission rate related to peripheral arterial disease (PAD) is rising, from approximately 480 per 100,000 US population in 2003 to 710 in 2011.[1] Simultaneously, an increasing proportion of patients undergo revascularization in an attempt to relieve symptoms or achieve limb preservation. Although there has been a national shift in revascularization procedures from open bypass to endovascular intervention, much controversy exists about the optimal initial management strategy for infrainguinal arterial disease.[2,3]

Evidence assessing the comparative effectiveness of both revascularization options has been hampered by the lack of randomized controlled trials, inclusion of previous ipsilateral failed procedures, and limited to small and regional retrospective series.[4-17] This is reflected by the lack of vascular society practice guidelines. While there is some consensus on the management for patients with claudication,[18] guidelines regarding the revascularization in chronic limb-threatening ischemia (CLTI) are still being developed in a collaboration between the Society of Vascular Surgery, the European Society of Vascular Surgery, and the World Federation of Vascular Societies, as part of a new global consortium. In the 2011, the American College of Cardiology Foundation/American Heart Association published guidelines on PAD and concluded that both treatment strategies result in comparable mortality and limb amputation rates up to 1 to 2 years, albeit dependent on anatomical patterns of disease and severity.[19] The lack of consensus is further illustrated by the absence of clear recommendations from the TASC II committee with regard to a preferred revascularization strategy,[20] which has precluded publication of the planned TASC III document. Overall, endovascular intervention has been associated with lower periprocedural morbidity, whereas other studies have suggested superior long-term clinical durability after bypass.[21] The majority of these studies, however, did not compare outcomes following first-time procedures, and included patients with failed prior ipsilateral treatment. This is problematic because several reports have suggested worse outcomes after repeated procedures.[22-24]

Besides disease severity and anatomical characteristics, optimal treatment strategies are also determined by the patients' general health status prior to revascularization. Although case selection is clearly applied, in particular for patients with preexisting conditions, well-defined patient characteristics that drive patient-centered decision-making are lacking.

Therefore, the purpose of our study was to identify nationwide patient characteristics associated with an endovascular-first approach, and to assess perioperative outcomes of patients undergoing first-time infrainguinal revascularization in a large-scale, national registry.

METHODS

Data source

This is a retrospective cohort study using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) targeted vascular module 2011-2014. As a national clinical registry, NSQIP aims to improve surgical care by providing risk-adjusted clinical outcomes in the first 30 days following the operative procedure. The targeted module greatly expands the variables collected and allows for more tailored, disease- and procedure-specific analyses. Clinical reviewers prospectively collect demographics, intraoperative details, and 30-day surgical outcomes in a standardized fashion according to NSQIP protocol. NSQIP methodology has been validated for data input accuracy and regular quality assessments are performed through internal and external audits.[25-27] All data within NSQIP have been de-identified and, therefore, approval was obtained from the Beth Israel Deaconess Medical Center Institutional Review Board and informed consent was waived.

Patients

All patients undergoing first-time infrainguinal bypass or endovascular intervention were used to create the cohort, excluding those with previous ipsilateral procedures. All emergent cases (N=316; 4.1%) and those undergoing hybrid procedures, namely concomitant bypass and endovascular interventions (N=204; 2.7%), were excluded. To appropriately compare the relative effectiveness of both treatment options, we additionally excluded patients admitted for a femoral-tibial/pedal bypass (N=1,152; 15%) because an equivalent multilevel endovascular procedure was only infrequently documented in NSQIP. The remaining cohort was stratified by symptom status (CLTI or claudication), after which we classified patients into procedure type, either isolated to the femoropopliteal or infrapopliteal segment. The type of revascularization was performed at the discretion of the treating physician. Stent placement during the endovascular procedure was not explicitly captured in this registry.

Variables

Demographics, intraoperative characteristics, and 30-day postoperative outcomes were compared between endovascular-first and bypass-first patients. Bypass conduit types were categorized by NSQIP into the following two groups: (i) single segment greater saphenous vein or (ii) prosthetic, arm vein or composite grafts. Postoperative outcomes within 30 days of the intervention included mortality, major adverse limb event (MALE), major adverse cardiovascular event (MACE), minor amputation (below ankle), wound complications, bleeding, renal deterioration, respiratory complications, unplanned return to the operating room, secondary revascularizations, and any unplanned readmission. MALE was considered when one of the following was documented: ipsilateral major amputation, major surgical graft revision, new bypass, or the use of thrombolysis or thrombectomy.[28] MACE was defined as postoperative myocardial infarction, stroke, or death of any cause. Bleeding was defined

as any transfusion or secondary procedure with the indication of bleeding. Secondary revascularizations included an ipsilateral new open bypass and surgical or endovascular revisions of the prior treated arterial segment. Renal deterioration refers to an increase in creatinine of 2 mg/dL relative to the last obtained value prior to the procedure, and/or requirement of dialysis. A respiratory complication included prolonged ventilator support (>48 hours), unplanned reintubation, or postoperative pneumonia. Discharge to home was not considered for those patients that were admitted from and subsequently discharged to a skilled or unskilled nursing facility. All variables including complication definitions were predefined by NSQIP prior to data collection and thus are not modifiable. These are publically accessible on: www.facs.org/quality-programs/acs-nsqip/program-specifics/participant-use.

Statistical analysis

For between-group comparisons, the Pearson's χ^2 or Fisher's exact test was performed for categorical variables, and the Student's t-test or Mann Whitney U test for continuous outcomes, where appropriate. Although we stratified baseline and intraoperative characteristics by procedure type (femoropopliteal vs. infrapopliteal) to provide greater detail specifically related to patient factors, in the remaining analyses these procedures are combined and adjusted for, due to the absolute low number of events for adjusted analysis. To identify current baseline characteristics of patients designated as undergoing an endovascular procedure as opposed to bypass surgery, we constructed multivariable logistic regression models stratified by symptom status. The predicted probability of undergoing an endovascular intervention was also determined, recognizing the additive effect of the obtained characteristics associated with this treatment modality. Additional multivariable logistic regression models were constructed to assess the independent associations between first-time procedures and 30-day outcomes. Covariates were selected using purposeful selection, incorporating backward selection after a univariate screen ($P < .10$) as well as including relevant patient factors previously identified.[29] Kaplan-Meier analysis was used to estimate unadjusted cumulative event rates and treatments were compared using the log-rank test. Statistical analyses were conducted with SPSS Statistics 23 (IBM Corp, Armonk, NY) and figures were generated using Prism 6 (GraphPad, La Jolla, CA). All tests were two-tailed and significance was considered when $P < .05$.

RESULTS

A total of 5,998 patients underwent first-time infrainguinal revascularization and were included in this study. In the CLTI cohort of 3,802 patients, 2,010 (53%) were treated with a bypass-first (19% of these were infrapopliteal procedures) and 1,792 (47%) with an endovascular-first approach (31% were infrapopliteal procedures). Among 2,196 patients with claudication, 1,183 (54%) underwent first-time bypass (5% were infrapopliteal procedures) and 1,013 (46%) first-time endovascular intervention (9% were infrapopliteal procedures).

Baseline characteristics in CLTI patients

Patient demographics and comorbidities were additionally stratified by procedure type and summarized in table I A (femoropopliteal) and I B (infrapopliteal). For both femoropopliteal and infrapopliteal procedures, CLTI patients undergoing first-time endovascular intervention were older, less commonly smokers, more likely functionally dependent, and more frequently suffered from preexisting conditions, such as diabetes, renal insufficiency, and current dialysis (all $P < .05$). Tissue loss was more common in CLTI patients treated with first-time endovascular intervention compared to bypass in both procedure types (femoropopliteal: 67% vs. 57%, $P < .001$; infrapopliteal: 86% vs. 79%, $P = .01$).

Baseline characteristics in patients with claudication

Among patients with claudication undergoing femoropopliteal or infrapopliteal procedures, those with first-time endovascular intervention were older, less likely to be white, and more frequently suffered from diabetes compared to first-time bypass (Table I A/B). In addition, among those with claudication and femoropopliteal disease, endovascular-first patients less commonly smoked and suffered from COPD, while they had more end-stage renal disease as compared to bypass-first patients (all $P < .05$).

Intraoperative details in CLTI patients

In patients with CLTI undergoing first-time bypass, single segment greater saphenous vein conduits were more frequently used than prosthetic, arm vein or composite grafts in femoropopliteal (56% vs. 44%) and infrapopliteal procedures (83% vs. 17%). As detailed in Table II A/B, CLTI patients treated with endovascular-first were less likely to undergo concurrent endarterectomy (femoropopliteal: 8.7% vs. 17%, $P < .001$; infrapopliteal: 1.4% vs. 5.9%, $P < .001$), and had shorter procedure times (femoropopliteal: 95 vs. 200 minutes, $P < .001$; infrapopliteal: 82 vs. 137 minutes, $P < .001$) than bypass-first patients.

Intraoperative details in patients with claudication

Of first-time bypass procedures, patients with claudication were more often revascularized with single segment greater saphenous vein conduits as opposed to prosthetic, arm vein or composite grafts in femoropopliteal (54% vs. 46%) and infrapopliteal procedures (77% vs. 23%). Endovascular-first patients had significantly shorter procedure times than bypass-first patients (femoropopliteal: 82 vs. 173 minutes, $P < .001$; infrapopliteal: 93 vs. 198 minutes, $P < .001$). Additional procedural differences are listed in Table II A/B.

Postoperative outcomes in CLTI patients

Compared to a bypass-first approach, the rate of 30-day mortality following first-time endovascular intervention was comparable among CLTI patients (2.1% vs. 2.2%, $P = .79$) (Table III). Similarly, MALE and major reintervention were equally distributed between both revascularization approaches (7.5% vs. 6.8%, $P = .43$ and 3.3% vs. 4.3%, $P = 12$, respectively). Although there was no significant difference in MACE, there was a trend

Table I A. Baseline characteristics and comorbidities in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing femoropopliteal procedures

	Femoropopliteal procedures									
	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=1636)		*(N=1233)*		**P-value**	*(N=1119)*		*(N=919)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Male gender	942	(58)	662	(54)	**.04**	740	(66)	586	(64)	.27
Age – years (SD)	68.4 (11)		70.1 (12)		**<.001**	66.0 (10)		67.8 (11)		**<.001**
Smoking	699	(43)	368	(30)	**<.001**	535	(48)	355	(39)	**<.001**
BMI > 30	469	(29)	368	(31)	.43	364	(33)	328	(36)	.10
Race					.81					**<.01**
White	1021	(62)	764	(62)		859	(77)	658	(72)	
Non-white	615	(38)	469	(38)		260	(23)	261	(28)	
Symptom status					**<.001**					
CLTI: rest pain	697	(43)	409	(33)						
CLTI: tissue loss	939	(57)	824	(67)						
Preoperative HCT ≤ 30%	265	(16)	245	(21)	**<.01**	48	(4.4)	31	(3.6)	.40
Not admitted from home	173	(11)	174	(14)	**<.01**	16	(1.4)	10	(1.1)	.49
Dependent functional status	159	(10)	175	(14)	**<.001**	12	(1.1)	16	(1.7)	.19
Hypertension	1357	(83)	1047	(85)	.16	894	(80)	762	(83)	.08
Diabetes	791	(48)	739	(60)	**<.001**	363	(32)	375	(41)	**<.001**
CHF	52	(3.2)	53	(4.3)	.11	14	(1.3)	15	(1.6)	.47
Renal Insufficiency	400	(25)	404	(34)	**<.001**	153	(14)	148	(17)	.09
Dialysis	142	(8.7)	165	(13)	**<.001**	12	(1.1)	20	(2.2)	**.046**
COPD	232	(14)	140	(11)	**.03**	168	(15)	102	(11)	**<.01**
Preprocedural medication										
Antiplatelet	1234	(76)	924	(76)	.98	905	(81)	776	(85)	**.04**
Statin	1090	(67)	792	(64)	.18	768	(69)	644	(71)	.37
Beta blocker	970	(60)	782	(64)	**.03**	588	(53)	487	(54)	.72

SD: standard deviation, *BMI*: body mass index, *HCT*: hematocrit, *CHF*: congestive heart failure, *COPD*: chronic obstructive pulmonary disease

toward lower cumulative rates after first-time endovascular intervention, particularly in the first 5 to 10 days post procedure (log-rank P = .07, Figure 1). When comparing first-time endovascular intervention to bypass, endovascular-first patients had significantly less surgical site infection (0.9% vs. 7.7%, P < .001), bleeding (8.5% vs. 17%, P < .001), respiratory complication (2.2% vs. 3.7%, P < .01), minor amputation (3.3% vs. 4.8%, P = .02), and a shorter total hospital stay (2 vs. 6 days, P < .001). While unplanned return to

the operating room was less frequently after first-time endovascular intervention (13% vs. 17%, P = .001), secondary bypass or endovascular procedures within 30 days were more common after first-time endovascular intervention (4.3% vs. 3.1%, P = .07), as was major amputation (4.6% vs. 3.3%, P = .04). Finally, CLTI patients treated with endovascular-first were more often discharged to home (86% vs. 69%, P < .001).

Table I B. Baseline characteristics and comorbidities in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing infrapopliteal procedures

	Infrapopliteal procedures									
	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=374)		*(N=559)*		**P-value**	*(N=64)*		*(N=94)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Male gender	267	(71)	371	(66)	.11	49	(77)	47	(50)	**.001**
Age – years (SD)	67.1 (12)		70.5 (12)		**<.001**	64.4 (14)		69.5 (12)		**.01**
Smoking	88	(24)	80	(14)	**<.001**	17	(27)	24	(26)	.89
BMI > 30	126	(34)	186	(35)	.88	21	(33)	34	(37)	.68
Race					.13					.06
White	220	(59)	301	(54)		50	(78)	60	(64)	
Non-white	154	(41)	258	(46)		14	(22)	34	(36)	
Symptom status					**.01**					
CLTI: rest pain	77	(21)	80	(14)						
CLTI: tissue loss	297	(79)	479	(86)						
Preoperative HCT ≤ 30%	99	(27)	152	(28)	.67	3	(4.8)	11	(12)	.16
Not admitted from home	63	(17)	107	(19)	.37	4	(6.3)	4	(4.3)	.72
Dependent functional status	41	(11)	112	(20)	**<.001**	2	(3.1)	3	(3.2)	>.99
Hypertension	315	(84)	486	(87)	.24	40	(63)	80	(85)	**.001**
Diabetes	249	(67)	407	(73)	**.04**	19	(30)	46	(49)	**.02**
CHF	15	(4.0)	32	(5.7)	.24	1	(1.6)	1	(1.1)	>.99
Renal Insufficiency	127	(34)	242	(44)	**<.01**	9	(14)	23	(26)	.08
Dialysis	40	(11)	120	(22)	**<.001**	3	(4.7)	4	(4.3)	>.99
COPD	30	(8.0)	46	(8.2)	.91	6	(9.4)	10	(11)	.80
Preprocedural medication										
Antiplatelet	279	(76)	435	(78)	.35	44	(70)	77	(83)	.06
Statin	232	(63)	353	(64)	.91	39	(64)	63	(68)	.63
Beta-blocker	230	(63)	371	(67)	.23	24	(39)	58	(62)	**<.01**

SD: standard deviation, *BMI*: body mass index, *HCT*: hematocrit, *CHF*: congestive heart failure, *COPD*: chronic obstructive pulmonary disease

Postoperative outcomes in patients with claudication

Among patients with claudication, mortality within 30 days did not differ significantly between endovascular-first and bypass-first patients (0.3% vs. 0.6%, P = .36), and neither did MALE (2.4% vs. 1.9%, P = .41) nor major amputation (0.3% vs. 0.3%, P > .99). Surgical site infections were less common following first-time endovascular intervention (0.7% vs. 6.6%, P < .001), while bypass-first patients demonstrated a constantly increasing cumulative rate of infection from postoperative day 5 to day 30 (Figure 1). Endovascular-first was additionally associated with lower rates of bleeding (2.3% vs. 6.0%, P < .001), respiratory complication (0.2% vs. 0.9%, P = .03), unplanned readmission (5.9% vs. 9.0%, P < .01), and a shorter total hospital stay (1 day vs. 3 days, P < .001) compared to bypass-first. Similar to CLTI patients, unplanned return to the operating room was less frequent in those with claudication after first-time endovascular intervention (4.3% vs. 6.6%, P = .02); however, secondary bypass or endovascular procedures occurred more often compared to first-time bypass (2.6% vs. 1.9%, P = .27) but this did not reach statistical significance. Unadjusted adverse event rates of both revascularization options stratified by procedure type are detailed in Supplementary table I A/B.

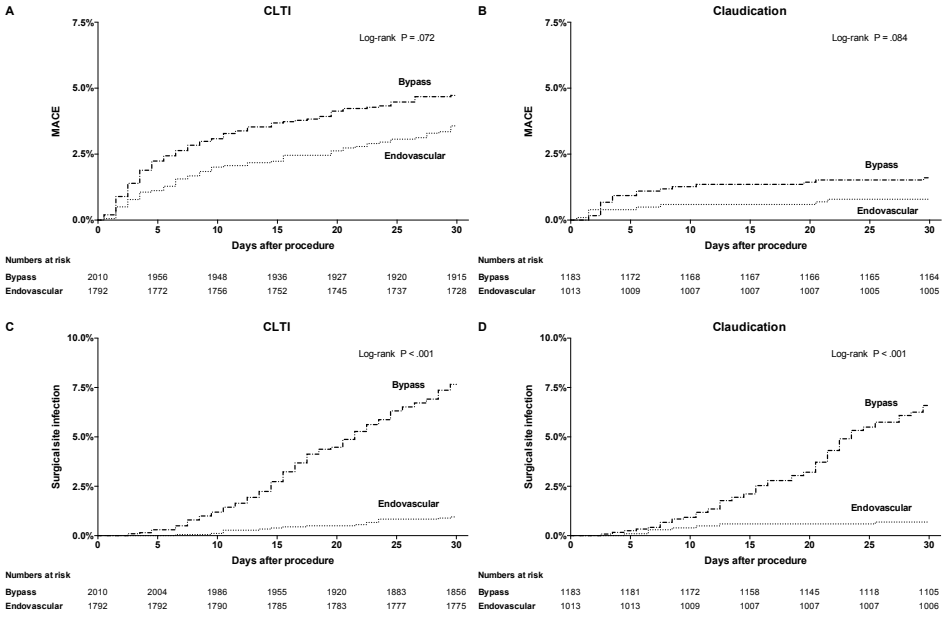

Figure 1. Cumulative adverse event rates during 30-day follow-up in patients with chronic limb-threatening ischemia (CLTI) and claudication for major adverse cardiovascular event (MACE) (**A.** and **B.**, respectively) and surgical site infection (**C.** and **D.**, respectively)

Multivariable analysis

With bypass-first as the reference group, we found comparable associations in patients with CLTI or claudication between an endovascular-first approach and several 30-day adverse outcomes (Table IV), which included: MACE (CLTI: odds ratio 0.6 [95% Confidence Interval 0.4-0.9]; claudication: OR 0.4 [0.2-0.95]), surgical site infection (CLTI: OR 0.1 [0.1-0.2]; claudication: OR 0.1 [0.04-0.2]), bleeding (CLTI: OR 0.4 [0.3-0.5]; claudication: OR 0.3 [0.2-0.5]), unplanned return to the operating room (CLTI: OR 0.7 [0.5-0.8]; claudication: OR

Table II A. Procedure details in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing femoropopliteal procedures

	Femoropopliteal procedures									
	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=1636)		*(N=1233)*		**P-value**	*(N=1119)*		*(N=919)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Concurrent procedures										
Endarterectomy	272	(17)	107	(8.7)	**<.001**	178	(16)	71	(7.7)	**<.001**
Elective procedure (vs. urgent)	1060	(65)	711	(58)	**<.001**	1054	(94)	848	(93)	.12
ASA class ≥ 4	399	(24)	221	(20)	**<.01**	132	(12)	70	(8.8)	**.03**
Procedure time – minutes (± IQR)	200 (150-267)		95 (67-137)		**<.001**	173 (128-238)		82 (59-120)		**<.001**

ASA: American Society of Anesthesiologists, *IQR*: interquartile range

Table II B. Procedure details in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing infrapopliteal procedures

	Infrapopliteal procedures									
	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=374)		*(N=559)*		**P-value**	*(N=64)*		*(N=94)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Concurrent procedures										
Endarterectomy	22	(5.9)	8	(1.4)	**<.001**	3	(4.7)	0	(0)	.07
Elective procedure (vs. urgent)	208	(56)	290	(52)	.27	53	(83)	77	(82)	.89
ASA class ≥ 4	108	(29)	111	(24)	.14	7	(11)	4	(6.9)	.54
Procedure time – minutes (± IQR)	243 (195-305)		92 (66-135)		**<.001**	198 (164-277)		93 (64-117)		**<.001**

ASA: American Society of Anesthesiologists, *IQR*: interquartile range

0.6 [0.4-0.9]), and unplanned readmission (CLTI: OR 0.8 [0.7-0.9]; claudication: OR 0.6 [0.4-0.8]). No significant association was found for mortality in CLTI patients (OR 0.7 [0.4-1.1]), while the death rate in those with claudication was too low to perform an adjusted analysis. Conversely, we found that endovascular-first was an independent predictor of ipsilateral secondary bypass or endovascular procedures within 30 days postoperatively for CLTI (OR: 1.6 [1.04-2.3]) but not for claudication (OR: 1.7 [0.9-3.4]).

Table III. Postoperative outcomes in patients with chronic limb-threatening ischemia (CLTI) and claudication in all procedures (femoropopliteal and infrapopliteal procedures combined)

	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=2010)		*(N=1792)*		**P-value**	*(N=1183)*		*(N=1013)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Mortality	44	(2.2)	37	(2.1)	.79	7	(0.6)	3	(0.3)	.36
MALE	137	(6.8)	134	(7.5)	.43	22	(1.9)	24	(2.4)	.41
Major amputation	67	(3.3)	83	(4.6)	**.04**	4	(0.3)	3	(0.3)	>.99
Major reintervention	87	(4.3)	60	(3.3)	.12	20	(1.7)	22	(2.2)	.41
Minor amputation	97	(4.8)	60	(3.3)	**.02**	7	(0.6)	4	(0.4)	.56
MACE	95	(4.7)	64	(3.6)	.08	19	(1.6)	8	(0.8)	.08
Surgical site infection	154	(7.7)	17	(0.9)	**<.001**	78	(6.6)	7	(0.7)	**<.001**
Wound dehiscence	37	(1.8)	4	(0.2)	**<.001**	14	(1.2)	1	(0.1)	**.001**
Bleeding	333	(17)	153	(8.5)	**<.001**	71	(6.0)	23	(2.3)	**<.001**
Renal deterioration	24	(1.2)	20	(1.1)	.82	6	(0.5)	1	(0.1)	.13
Requiring dialysis	13	(0.6)	6	(0.3)	.17	2	(0.2)	1	(0.1)	>.99
Respiratory complication	74	(3.7)	39	(2.2)	**<.01**	11	(0.9)	2	(0.2)	**.03**
Unplanned return to the OR	346	(17)	240	(13)	**.001**	78	(6.6)	44	(4.3)	**.02**
Secondary revascularization	53	(3.1)	59	(4.3)	.07	19	(1.9)	20	(2.6)	.27
Untreated loss of patency	36	(1.8)	25	(1.4)	.33	9	(0.8)	8	(0.8)	>.99
Discharge to home	1301	(69)	1423	(86)	**<.001**	1076	(92)	992	(99)	**<.001**
Length of hospital stay – days (± IQR)	6 (4-12)		2 (1-8)		**<.001**	3 (2-5)		1 (0-1)		**<.001**
Unplanned readmission	352	(18)	299	(17)	.50	107	(9.0)	60	(5.9)	**<.01**

MALE: major adverse limb event, *MACE*: major adverse cardiovascular event, *OR*: operating room, *IQR*: interquartile range

Table IV. Adjusted associations between first-time revascularizations and postoperative outcomes in patients with chronic limb-threatening ischemia (CLTI) and claudication

	Endovascular-first strategy (vs. Bypass-first)					
	CLTI [a]			**Claudication [b]**		
30-day outcomes	*OR*	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
Mortality	0.7	0.4-1.1	.12	*		
MALE	1.0	0.8-1.3	.89	1.3	0.7-2.4	.40
Major amputation	1.1	0.8-1.6	.58	*		
MACE	0.6	0.4-0.9	**<.01**	0.4	0.2-0.95	**.04**
Surgical site infection	0.1	0.1-0.2	**<.001**	0.1	0.04-0.2	**<.001**
Bleeding	0.4	0.3-0.5	**<.001**	0.3	0.2-0.5	**<.001**
Unplanned return to the operating room	0.7	0.5-0.8	**<.001**	0.6	0.4-0.9	**.03**
Secondary revascularization	1.6	1.04-2.3	**.03**	1.7	0.9-3.4	.13
Unplanned readmission	0.8	0.7-0.9	**<.01**	0.6	0.4-0.8	**.001**

OR: odds ratio, *CI*: confidence interval, *MALE*: major adverse limb event, *MACE*: major adverse cardiovascular event. [a] adjusted for age, gender, tissue loss, race, smoking, hypertension, diabetes, congestive heart failure, renal insufficiency, preoperative dialysis, chronic obstructive pulmonary disease, type of procedure, dependent functional status, elective procedure. [b] adjusted for age, gender, race, smoking, diabetes, renal insufficiency, type of procedure

* Too few events

Current patient selection

Independent factors associated with an endovascular-first as opposed to a bypass-first approach are displayed in Figure 2. Patient characteristics that could predict first-time endovascular intervention as a treatment modality in CLTI patients were age ≥80 years, tissue loss, non-smoking, functional dependence, diabetes, current dialysis, and infrapopliteal lesions. Current factors associated with an endovascular intervention in patients with claudication included age ≥80 years, non-white race, non-smoking, diabetes, and infrapopliteal lesions. The effect of these acquired factors was additive, with a predicted probability of being treated with an endovascular intervention of 62% in CLTI patients and 72% in those with claudication when 4 or more of these patient characteristics were present (Figure 3).

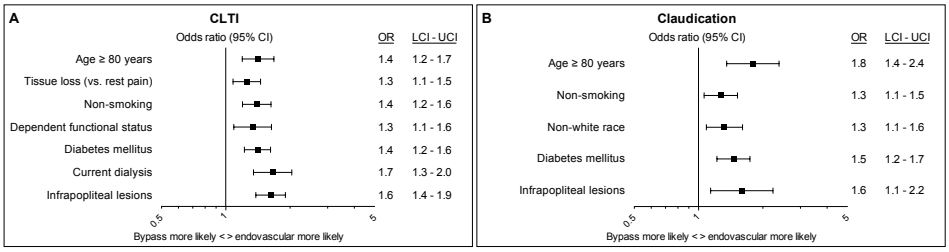

Figure 2. Patient characteristics associated with an endovascular-first approach in **A.** chronic limb-threatening ischemia (CLTI) and **B.** claudication

CI: Confidence interval, *OR*: odds ratio, *LCI*: lower confidence interval, *UCI*: upper confidence interval
Models adjusted additionally for gender

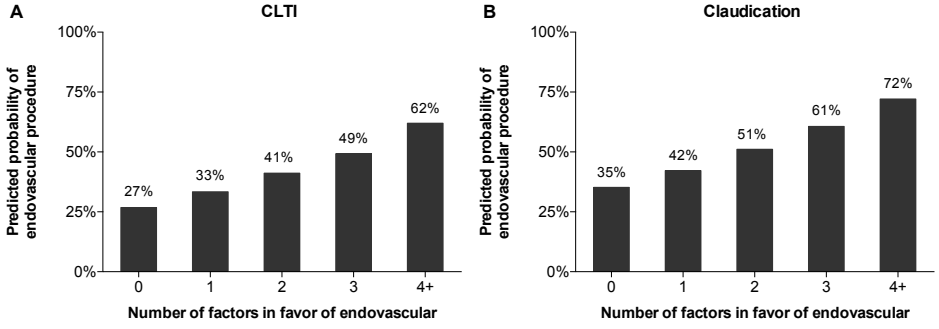

Figure 3. Predicted probability of undergoing a first-time endovascular intervention by the number of associated factors identified in patients with **A.** chronic limb-threatening ischemia (CLTI) and **B.** claudication

DISCUSSION

This study demonstrates that 30-day morbidity, but not mortality, of patients treated with first-time endovascular intervention for infrainguinal arterial disease is substantially lower than that of bypass-first patients, despite having more comorbidities. The endovascular treatment modality, however, was independently associated with higher rates of ipsilateral secondary revascularizations within 30 days, as these were one-and-a-half times more likely among patients with CLTI. In addition, in both patients with CLTI and claudication, factors associated with an endovascular-first approach included patient demographics and anatomic characteristics, as well as more severe, PAD-related comorbidities such as diabetes and end-stage renal disease.

To date, only two randomized controlled trials have attempted to address the comparative effectiveness of both revascularization strategies in infrainguinal disease[4,5], yet these trials include previous ipsilateral interventions and are already considered outdated due to evolving endovascular techniques. The most prominent trial, the BASIL trial, was conducted between 1999-2004 in the United Kingdom and compared infrainguinal bypass to balloon angioplasty alone in 452 patients with severe limb ischemia.[4] Ultimately, the BASIL trial demonstrated no difference in mortality or amputation-free survival up to 2 years; however, in patients surviving more than 2 years, survival was higher in those who had bypass.[30] Thirty-day mortality was comparable, with 5% in the bypass and 3% in the angioplasty group, while bypass was associated with significantly higher morbidity (57% vs. 41%).[4] The event rates were substantially higher than the current study, most likely related to several issues in study design, including no use of stents or drug-coated balloons in the angioplasty treatment group as well as the detrimental effects of repeated procedures, which limits the widespread applicability to first-time procedures. Although upcoming randomized trials, the BASIL 2 and BEST-CLI, will likely provide greater detail with respect to both treatment approaches, results will not be published for several years.[31,32]

Since the above-mentioned trials lack robust efficacy data and limit real-world generalizability, population-based observational studies may provide some guidance and new insights. In retrospective studies there is a general consensus, reflected by a recent Vascular Quality Initiative (VQI) study, which demonstrated that, in 7,897 revascularizations performed for CLTI, endovascular intervention was independently associated with lower 30-day mortality (OR 0.6 [0.4-0.8]) but worse 3-year survival (hazard ratio 1.2 [1.1-1.4]) as compared to bypass surgery.[7] Similarly, Tsai et al. reported in 1,858 patients from community-based registries in Colorado and California that, using propensity analyses, those with CLTI or claudication treated with endovascular intervention were less likely to die within 30 days as compared to bypass (0.2% vs. 3.8%).[8] However, contrary to the aforementioned study, patients who underwent endovascular intervention showed better survival rates as opposed to bypass over a follow-up period of 3 years. Our study may contribute to the current literature by demonstrating no differences in 30-day survival as opposed to the previous studies, which may have important clinical implications for first-time interventions since all prior studies included repeated procedures. Evidently, these prior procedures are impacting short- and long-term outcomes, which is supported by recent cohort studies.[22-24]

There have been a handful of studies directly comparing first-time revascularizations.[11-13,15] While limited by small sample sizes and single institution data, these studies also demonstrated comparable mortality and limb salvage data for both treatment approaches in patients with CLTI and claudication. Thirty-day morbidity was additionally similar between first-time endovascular and bypass, which is likely a result of low power in the majority of reports. Our recent single institution study, which included 1,336 first-time procedures for CLTI, also demonstrated that mortality (2.8% vs. 3.3%) and transmetatarsal amputations

(5% vs. 4%) did not differ between endovascular-first and bypass-first patients, although the unadjusted and adjusted 3-year survival as well as freedom from restenosis and reintervention were better for bypass-first patients.[13] As a national representation of first-time infrainguinal interventions, this study may function as a useful adjunct to patient-centered decision-making by weighing risks and benefits of perioperative morbidity against reinterventions in patients with multiple comorbidities. A comparison of adverse postoperative events between first-time endovascular intervention and bypass is complicated by the prevalence and diversity of preexisting conditions. However, as length of stay was shorter, as well as the incidence of postoperative complications (e.g., cardiac and wound complications) and unplanned readmissions were lower among those undergoing endovascular intervention despite comorbidities being more pronounced, this study further supports the benefits of first-time endovascular intervention over bypass in the perioperative period and for those with a relatively short life expectancy.

Factors used to select the method of revascularization are heterogeneous, may differ per institution and physician, but may also change over time with increased experience and adoption of novel techniques and approaches. The present study determined current nationwide patient selection for first-time endovascular intervention in the United States and we found that older patients, as well as those with more severe comorbidities and infrapopliteal disease were more likely candidates to undergo an endovascular-first approach. These patients presumably represent a selected group who were less suitable for open surgery or have lesion-related characteristics that may compromise its success. One other retrospective cohort study used patient characteristics to predict the probability of undergoing revascularization in 1,200 German patients with CLTI.[16] The authors concluded that the probability of being treated with bypass was noticeably higher in those with normal renal function, current smokers, previous failed interventions, TASC C or D lesions, and three run-off vessels. Although the current study found, in part, similar characteristics, NSQIP lacks specificity such as detailed anatomic and lesion data and, therefore, confounding by indication may influence these results. However, awareness of these patient characteristics is relevant to both the physician and patient for identifying the optimal type of treatment and to develop realistic expectations based on nationwide experiences.

There are some additional limitations to this study. As a retrospective study, the potential for treatment selection bias exists since our data were subject to provider experience and preferences. Furthermore, the targeted vascular module consists of a randomly selected subset of patients from participating hospitals nationwide and, as a result, the proportion of patients undergoing one of both revascularization approaches may not be representative of all practices. Despite adjusting for a number of covariates in our multivariable models, within NSQIP we were limited by predefined variables recorded in this registry as well as unmeasured confounders, such as extent of arterial lesion and wound severity. In addition, 5% of patients with claudication in our cohort underwent an infrapopliteal bypass, and while this remains a controversial topic, it is possible that the

symptom status of some of these patients was miscoded. Finally, although we attempted to account for extent of disease by separating femoropopliteal and infrapopliteal disease, lesion data would have added great detail to our patient selection model and comparison of both treatment strategies.

CONCLUSIONS

This study demonstrates the early morbidity – but not mortality – benefit of endovascular intervention over bypass in first-time infrainguinal revascularizations, although more postoperative secondary revascularizations were performed after an endovascular-first approach. As more patients undergo revascularization, there remains a lack of evidence to support either approach having a clear advantage over the other. Accepting these limitations, this study highlights the early perioperative benefits of an endovascular-first strategy, but more robust data on long-term outcomes in select PAD patients are needed before this can be recommended as the therapy of choice.

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SUPPLEMENTARY

Supplementary table I A. Postoperative outcomes in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing femoropopliteal procedures

	Femoropopliteal procedures									
	CLTI					Claudication				
	Bypass - first		Endovascular - first			Bypass - first		Endovascular - first		
	(N=1636)		(N=1233)		P-value	(N=1119)		(N=919)		P-value
	N	%	N	%		N	%	N	%	
Mortality	36	(2.2)	31	(2.5)	.58	6	(0.5)	3	(0.3)	.53
MALE	114	(7.0)	81	(6.6)	.67	19	(1.7)	22	(2.4)	.27
Major amputation	56	(3.4)	43	(3.5)	.93	2	(0.2)	2	(0.2)	>.99
Major reintervention	74	(4.5)	43	(3.5)	.17	18	(1.6)	21	(2.3)	.27
Minor amputation	69	(4.2)	38	(3.1)	.11	4	(0.4)	2	(0.2)	.70
MACE	74	(4.5)	54	(4.4)	.85	18	(1.6)	7	(0.8)	.08
Surgical site infection	140	(8.6)	11	(0.9)	**<.001**	76	(6.8)	7	(0.8)	**<.001**
Wound dehiscence	33	(2.0)	3	(0.2)	**<.001**	14	(1.3)	1	(0.1)	**<.01**
Bleeding	251	(15)	111	(9.0)	**<.001**	64	(5.7)	22	(2.4)	**<.001**
Renal deterioration	22	(1.3)	14	(1.1)	.62	5	(0.4)	1	(0.1)	.23
Requiring dialysis	13	(0.8)	2	(0.2)	**.02**	2	(0.2)	1	(0.1)	>.99
Respiratory complications	54	(3.3)	22	(1.8)	**.01**	11	(1.0)	2	(0.2)	**.046**
Unplanned return to the OR	273	(17)	141	(11)	**<.001**	70	(6.3)	37	(4.0)	**.03**
Secondary revascularization	48	(3.4)	42	(4.4)	.22	17	(1.8)	17	(2.4)	.35
Untreated loss of patency	28	(1.7)	13	(1.1)	.14	5	(0.4)	8	(0.9)	.23
Discharge to home	1085	(70)	986	(86)	**<.001**	1026	(92)	902	(99)	**<.001**
Length of hospital stay – days (± IQR)	6 (3-11)		2 (1-7)		**<.001**	3 (2-5)		1 (0-1)		**<.001**
Unplanned readmission	284	(17)	185	(15)	.09	100	(8.9)	51	(5.5)	**<.01**

MALE: major adverse limb event, *MACE*: major adverse cardiovascular event, *OR*: operating room, *IQR*: interquartile range

Supplementary table I B. Postoperative outcomes in patients with chronic limb-threatening ischemia (CLTI) and claudication undergoing infrapopliteal procedures

	Infrapopliteal procedures									
	CLTI					**Claudication**				
	Bypass - first		**Endovascular - first**			**Bypass - first**		**Endovascular - first**		
	(N=374)		*(N=559)*		**P-value**	*(N=64)*		*(N=94)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Mortality	8	(2.1)	6	(1.1)	.19	1	(1.6)	0	(0)	.41
MALE	23	(6.1)	53	(9.5)	.07	3	(4.7)	2	(2.1)	.40
Major amputation	11	(2.9)	40	(7.2)	**<.01**	2	(3.1)	1	(1.1)	.57
Major reintervention	13	(3.5)	17	(3.0)	.71	2	(3.1)	1	(1.1)	.57
Minor amputation	28	(7.5)	22	(3.9)	**.02**	3	(4.7)	2	(2.1)	.40
MACE	21	(5.6)	10	(1.8)	**.001**	1	(1.6)	1	(1.1)	>.99
Surgical site infection	14	(3.7)	6	(1.1)	**<.01**	2	(3.1)	0	(0)	.16
Wound dehiscence	4	(1.1)	1	(0.2)	.16	0	(0)	0	(0)	-
Bleeding	82	(22)	42	(7.5)	**<.001**	7	(11)	1	(1.1)	**<.01**
Renal deterioration	2	(0.5)	6	(1.1)	.49	1	(1.6)	0	(0)	.41
Requiring dialysis	0	(0)	4	(0.7)	.15	0	(0)	0	(0)	-
Respiratory complications	20	(5.3)	17	(3.0)	.08	0	(0)	0	(0)	-
Unplanned return to the OR	73	(20)	99	(18)	.49	8	(13)	7	(7.4)	.28
Secondary revascularization	5	(1.7)	17	(4.2)	.06	2	(4.0)	3	(5.6)	.71
Untreated loss of patency	8	(2.1)	12	(2.1)	.99	4	(6.3)	0	(0)	**.03**
Discharge to home	216	(64)	437	(86)	**<.001**	50	(80)	90	(98)	**<.001**
Length of hospital stay – days (± IQR)	9 (5-15)		3 (1-9)		**<.001**	4 (3-7)		0 (0-1)		**<.001**
Unplanned readmission	68	(18)	114	(20)	.40	7	(11)	9	(9.6)	.78

MALE: major adverse limb event, *MACE*: major adverse cardiovascular event, *OR*: operating room, *IQR*: interquartile range

CHAPTER 3

OUTCOMES OF LOWER EXTREMITY REVASCULARIZATION BETWEEN PATIENTS WITH AND WITHOUT DIABETES

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ABSTRACT

Objective

Data on the effect of diabetes type (insulin-dependent vs. noninsulin- dependent) on short- and long-term outcomes after lower extremity revascularization for chronic limb-threatening ischemia (CLTI) are lacking. We sought to address this paucity of information by evaluating outcomes in patients with insulin-dependent and noninsulin-dependent diabetes after first-time bypass and endovascular interventions.

Methods

We reviewed all limbs undergoing a first-time infrainguinal bypass (BPG) or percutaneous transluminal angioplasty with or without stent (PTA/S) for CLTI at our institution from 2005-2014. Based on preoperative medication regimen, patients were categorized as having insulin-dependent diabetes (IDDM), noninsulin-dependent diabetes (NIDDM), or no diabetes (NDM). Outcomes included wound healing, major amputation, RAS events (revascularization, major amputation, or stenosis), major adverse limb events (MALE), and mortality. Outcomes were evaluated using Chi-square, Kaplan-Meier, and Cox regression analyses.

Results

Of 2,869 infrainguinal revascularizations from 2005-2014, 1,294 limbs (646 BPG, 648 PTA/S) fit our criteria and underwent a first-time revascularization for CLTI. Overall, 703 IDDM, 262 NIDDM, and 329 NDM limbs were included in our analysis. IDDM patients, compared to NIDDM and NDM, were younger (69 vs. 73 vs. 77 years; P<.001) and more often presented with tissue loss (89% vs. 77% vs. 67%; P<.001), coronary artery disease (57% vs. 48% vs. 43% P<.001), and end-stage renal disease (26% vs. 13% vs. 12%; P<.001). Perioperative complications, including mortality (3% vs. 2% vs. 5%; P=.07), did not differ between the three groups; however, complete wound healing at 6-month follow-up was significantly worse among IDDM patients (36% vs. 40% vs. 51%; P<.001). Irrespective of intervention type, IDDM patients had significantly higher three-year major amputation rates (BPG: 24% vs. 16% vs. 10%, P=.04; PTA/S: 21% vs. 6% vs. 5%, P<.001). Multivariable analyses illustrated that, compared to NDM, IDDM was associated with significantly higher risk of both major amputation and RAS events following any first-time intervention (Hazard Ratio (HR) 2.5, 95% Confidence Interval [CI] 1.2-5.2 and 1.4 [1.1-1.9], respectively). Similar associations were found for a PTA/S-first intervention (3.1 [1.1-9.0] and 1.5 [1.1-2.2], respectively), while IDDM patients undergoing a BPG-first intervention were only associated with incomplete wound healing (1.5 [1.3-2.9]). Lastly, when compared to NDM, NIDDM was associated with lower late mortality (0.6 [0.5-0.8]).

Conclusions

As compared to NDM, IDDM was associated with similar perioperative and long-term mortality but a higher risk of incomplete wound healing, major amputation, and future RAS events, especially after a PTA/S-first approach. Interestingly, NIDDM was associated with lower long-term mortality and not associated with any adverse limb events. Overall, these data demonstrate both the importance in distinguishing between diabetes types, as well as potential long-term benefit of a bypass-first strategy in appropriately selected in IDDM patients with CLTI.

INTRODUCTION

Despite advances in the management of diabetes, the profound effect of the estimated growth is still likely to yield a tremendous escalation in end-stage peripheral arterial disease (PAD).[1] Chronic limb-threatening ischemia (CLTI), broadly defined as the most advanced stages of PAD and demarcated by ischemic rest pain, non-healing ulcer, or gangrene, is significantly more likely in diabetic patients and is often a debilitating condition.[2] Ultimately, the diagnosis of PAD in patients with diabetes is often delayed due to presence of neuropathy, as PAD-related symptoms go unnoticed until more severe CLTI symptoms develop.[3] Given the prevalence and severity of such events, non-operative wound management and care may not be sufficient to avoid limb loss.

Although open surgical bypass (BPG) has been shown to have excellent results in patients with diabetes and PAD, contemporary management of CLTI has gradually favored the use of minimally invasive techniques that offer lower periprocedural morbidity and mortality, reduced costs, faster procedural times, and a shortened hospital stay.[4] Several studies have compared the utility of both BPG and percutaneous transluminal angioplasty with or without stenting (PTA/S) in varying degrees of lower extremity limb ischemia, and in patients with and without diabetes; however, in the current endovascular era, few studies have evaluated the degree to which these subsets of patients fare in regard to procedure type.[5-13] In this study, we sought to describe our institution's long-term experience with BPG-first and PTA/S-first repair in insulin-dependent, noninsulin-dependent, and non-diabetic patients.

METHODS

Subjects and settings

We performed a retrospective review of all patients with CLTI undergoing a first-time lower extremity intervention at Beth Israel Deaconess Medical Center (BIDMC). Medical records of all BPG and PTA/S interventions from January 2005 to October 2014 were individually reviewed. Patients were categorized as having insulin-dependent diabetes (IDDM), noninsulin-dependent diabetes (NIDDM), or no diabetes (NDM). IDDM was defined as reliance on insulin administration at baseline to control diabetes. Patients who had diabetes, but were not prescribed any insulin, were categorized as having NIDDM. Importantly, insulin dependence within this study is not synonymous with type I diabetes, but rather defines the patient-level pattern of insulin utilization at the time of revascularization. Patients who received previous interventions on the ipsilateral limb (whether at BIDMC or at an outside institution) or interventions at or proximal to the iliac arteries were excluded. Patients undergoing a concomitant procedure, including endarterectomy, profundaplasty, thrombectomy, atherectomy, or patch, were included and adjusted for in our analyses. Typical follow-up interval and modality was every 3 to 4 months for 2 years and every

6 months thereafter with arterial duplex ultrasound imaging and ankle-brachial indices with forefoot pulse volume recordings and/or toe pressures. Re-intervention was performed for symptomatic graft restenosis or threatened asymptomatic grafts (peak systolic velocity ratio >3.5–4 or low graft velocities <30cm/second). Generally, patients did not undergo re-interventions for an asymptomatic restenosis after angioplasty alone; however, we were more likely to re-intervene with PTA/S for an asymptomatic in-stent restenosis if the peak systolic velocity ratio was >3.5–4. We were less likely to re-intervene percutaneously for a symptomatic restenosis if the disease was extensive and restenosis was rapid, as we feel this has a low likelihood of deriving a durable benefit.

We included patients whose disease severity was distinctly identifiable as CLTI and who underwent either BPG or PTA/S. Indications for intervention included tissue loss (i.e., gangrene and ulcer) or rest pain, where patients presenting with more than one indication for intervention were assigned according to the following hierarchy: gangrene, ulcer, then rest pain. Femoropopliteal lesion anatomy and severity were defined according to the modified Trans-Atlantic Inter-Society Consensus (TASC II) classification, while tibial lesion information was defined by TASC I, as no updated TASC class for tibial lesions was included in the modified TASC II.[14, 15]

Measurements and outcome variables

Primary outcomes included perioperative complications, wound healing, major amputation, RAS events (a composite variable denoted by re-intervention, major amputation, or stenosis), major adverse limb events (MALE, a composite variable denoted by any major amputation or any major re-intervention, defined as creation of a new bypass graft, a jump/interposition graft revision, surgical thrombectomy with or without surgical patch angioplasty, and thrombectomy of an occluded graft or arterial segment using pharmacologic or mechanical thrombolysis), and mortality.[16] Demographics, comorbidities, restenosis, and re-intervention were also recorded. Perioperative complications included hematoma, acute myocardial infarction, and death. Criteria for restenosis was at least >75% stenosis by angiographic measurement, or a >3.5 fold increase in peak systolic velocity by duplex. Re-intervention included any ipsilateral surgical or endovascular revision. The decision of intervention type was surgeon-dependent and varied over time with the acquisition of endovascular skills: In general, primary angioplasty with selective stenting was done at the clinical judgment of the attending physician at the time of the angiogram. Routine statin use was introduced over time. PTA/S patients were generally discharged on clopidogrel for one month post-operatively and aspirin indefinitely. Technical success for PTA/S was defined as less than 30% residual stenosis and no flow-limiting dissection. Following a bypass procedure, a patent graft at completion, no significant defect in the vein on angioscopy, and continuous wave Doppler interrogation were required for technical success.

Statistical Analyses

Contingent on the outcome of interest, analyses were performed on either a per-limb basis (i.e., wound healing, stenosis, re-intervention, amputation, RAS, MALE) or a per-patient basis (i.e., mortality), where, on per-patient outcomes, the initial limb was censored at the procedure date of the contralateral limb. Pearson chi-square and Fisher exact tests were used for comparisons of categorical variables. Continuous variables were compared using Student t-test or Mann-Whitney U test, as appropriate. Rates were compared across strata (IDDM, NIDDM, and NDM) using chi-square. Treatment outcomes during the course of follow-up were analyzed using Kaplan-Meier methodology, and unadjusted time-to-failure curves were compared with the log-rank test. Covariates were selected using purposeful selection, incorporating backward selection after a univariate screen ($P < .10$) as well as including relevant patient factors previously identified.[17] Multivariable Cox regression models were constructed to assess independent associations between diabetes type and time-dependent outcomes. Statistical significance was defined as $P < .05$. All statistical tests were done using STATA 13 (StataCorp, College Station, Tex). The Beth Israel Deaconess Medical Center Institutional Review Board approved this study and waived the need for patient consent.

RESULTS

Baseline Characteristics

Of the 2,869 total lower extremity revascularizations performed between January 2005 and October 2014, 1,294 limbs in 1,160 patients met our inclusion criteria (i.e., a first-time lower extremity intervention for CLTI with reliable insulin information): 648 undergoing a primary BPG and 646 undergoing a primary PTA/S. As Figure 1 A illustrates, the number of IDDM limbs treated with a revascularization gradually decreased over the study period (from 84 procedures in 2005 to 58 in 2013), as did the number of IDDM limbs treated with a BPG-first approach (from 79% in 2005 to 22% in 2013). Additionally, as Figure 1 B and 1 C demonstrate, these decreasing trends remained relatively consistent across NIDDM and NDM limbs, with the former undergoing 61% BPG-first procedures in 2005 and 26% in 2013 and the latter falling from 71% BPG-first interventions to 22%.

Overall, 703 IDDM, 262 NIDDM, and 329 NDM limbs were included in our analysis. IDDM patients, compared to NIDDM and NDM patients, respectively, were younger (69 vs. 73 vs. 77 years; $P < .001$) and more often presented with tissue loss (89% vs. 78% vs. 67%; $P < .001$), coronary artery disease (57% vs. 48% vs. 43% $P < .001$), and end-stage renal disease (26% vs. 13% vs. 12%; $P < .001$) (Table I). Conversely, NDM patients more commonly suffered from COPD (10% vs. 9% vs. 19%; $P < .001$) and more frequently smoked (57% vs. 58% vs. 69%; $P = .001$). Groups did not differ in male sex (62% vs. 57% vs. 56%; $P = .13$) or in rates of congestive heart failure (34% vs. 28% vs. 28%; $P = .10$).

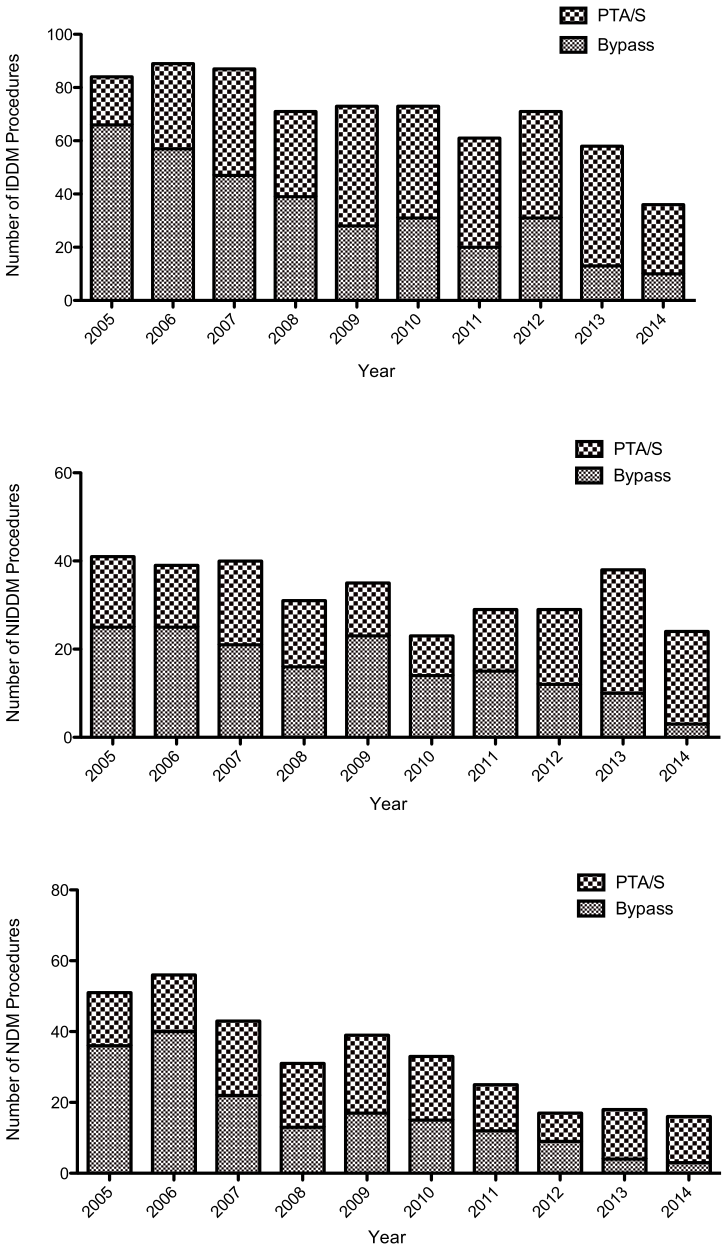

Figure 1. Number of yearly first-time revascularization procedures performed on patients with chronic limb-threatening ischemia and **A**. insulin-dependent diabetes (IDDM), **B**. noninsulin-dependent diabetes (NIDDM), and **C**. no diabetes (NDM)

PTA/S: percutaneous angioplasty with or without stent, *IDDM:* insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes

Table I. Demographics, co-morbidities, and pre-operative lesion characteristics between 1,294 patients with insulin-dependent diabetes, noninsulin-dependent diabetes, and no diabetes with chronic limb-threatening ischemia (CLTI)

	IDDM *(N=703)*	**NIDDM** *(N=262)*	**NDM** *(N=329)*	**P-value** (IDDM to NIDDM)	**P-value** (IDDM to NDM)	**P-value** (NIDDM to NDM)	**P-value**
Demographics No. (%)							
Age, mean (SD)	68.9 (12.0)	72.9 (11.0)	76.6 (12.5)	**<.001**	**<.001**	**<.001**	**<.001**
Race				**.03**	**<.001**	**.04**	**<.001**
White	518 (74)	210 (81)	286 (87)				
Non-white	182 (26)	50 (19)	43 (13)				
Male sex	436 (62)	148 (57)	185 (56)	.13	.08	.91	.13
Indication, No. (%)							
Rest Pain	78 (11)	59 (23)	108 (33)	**<.001**	**<.001**	**<.01**	**<.001**
Ulcer	407 (58)	124 (47)	148 (45)	**<.01**	**<.001**	.57	**<.001**
Gangrene	218 (31)	79 (30)	73 (22)	.80	**<.01**	**.03**	**.01**
Comorbidities, No. (%)							
Coronary artery disease	395 (57)	123 (48)	136 (43)	**.01**	**<.001**	.27	**<.001**
Hypertension	622 (89)	226 (87)	242 (76)	.47	**<.001**	**.001**	**<.001**
Hyperlipidemia	466 (67)	145 (56)	167 (53)	**<.01**	**<.001**	.43	**<.001**
Dialysis dependence	185 (26)	34 (13)	38 (12)	**<.001**	**<.001**	.67	**<.001**
BMI, mean	29.0	27.6	24.5	**<.01**	**<.001**	**<.001**	**.04**
History of myocardial infarction	215 (31)	65 (25)	54 (17)	.09	**<.001**	**.02**	**<.001**
Congestive heart failure	234 (34)	73 (28)	88 (28)	.12	.06	.89	.10
COPD	69 (10)	23 (8.9)	60 (19)	.66	**<.001**	**.001**	**<.001**
Smoking history	401 (57)	150 (58)	220 (69)	.88	**<.001**	**<.01**	**.001**
WIfI clinical stage,[16] No. (%)							
Clinical stage 1	6 (1.2)	2 (1.0)	3 (1.2)	.85	.98	.85	.98
Clinical stage 2	99 (19)	65 (33)	116 (46)	**<.001**	**<.001**	**<.01**	**<.001**
Clinical stage 3	140 (27)	48 (24)	56 (22)	.37	.13	.66	.28
Clinical stage 4	268 (52)	85 (43)	77 (31)	**.02**	**<.001**	**<.01**	**<.001**
Fempop TASC classification, No. (%)							
TASC A	120 (19)	50 (22)	42 (15)	.49	.11	.05	.13
TASC B	185 (30)	78 (34)	82 (29)	.30	.83	.28	.50
TASC C	76 (12)	30 (13)	44 (16)	.80	.17	.38	.38
TASC D	104 (17)	44 (19)	88 (31)	.46	**<.001**	**.001**	**<.001**
Tibial TASC classification, No. (%)							
TASC A	78 (13)	30 (13)	35 (13)	.83	.97	.87	.98
TASC B	143 (23)	46 (20)	55 (20)	.36	.29	.95	.46
TASC C	138 (23)	50 (21)	55 (20)	.91	.43	.58	.72
TASC D	198 (32)	66 (29)	87 (32)	.40	.89	.53	.70

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM:* non-diabetes, *BMI:* body mass index, *COPD:* chronic obstructive pulmonary disease, *WIfI:* wound, ischemia, and foot infection, *TASC:* Trans-Atlantic Inter-Society Consensus

There was no difference in the proportion of patients undergoing a primary BPG by diabetes type (49% vs. 51% vs. 52%; P = .58). The prevalence of preoperative femoropopliteal TASC D lesions was lowest in IDDM patients (17% vs. 19% vs. 31%; P < .001), although this difference was not seen when directly comparing IDDM to NIDDM (P = .46). There was no difference in tibial TASC D lesions (32% vs. 29% vs. 32%; P = .69). Finally, WIfI clinical stage 4 was most prevalent among IDDM patients (52% vs. 43% vs. 31%; P < .02), potentially driven by the high WIfI wound component among these patients (1.6 vs. 1.4 vs. 1.2; P < .01).[18]

Of the 646 BPG-first procedures, the femoral artery was the most common inflow artery (74% of all procedures), although significantly less so among IDDM patients (68% vs. 74% vs. 84%; P = .001). When directly comparing IDDM to NDM, the outflow artery among IDDM patients was less commonly the popliteal artery (29% vs. 40%; P = .01) and was more commonly the dorsalis pedis/pedal arteries (29% vs. 16%; P < .01) (Table II). Procedural

Table II. Operative details of 646 insulin-dependent, noninsulin-dependent, and non-diabetic patients undergoing open surgical bypass for chronic limb-threatening ischemia (CLTI)

	IDDM *(N=342)*	**NIDDM** *(N=133)*	**NDM** *(N=171)*	**P-value** (IDDM to NIDDM)	**P-value** (IDDM to NDM)	**P-value** (NIDDM to NDM)	**P-value**
Inflow artery, No. (%)							
Femoral	233 (68)	99 (74)	143 (84)	.18	**<.001**	**.049**	**.001**
Popliteal	109 (32)	34 (26)	27 (16)	.18	**<.001**	**.04**	**<.001**
Tibial	1 (0.6)	0 (0)	0 (0)	.16	.38	-	.25
Outflow artery, No. (%)							
Popliteal	98 (29)	45 (34)	69 (40)	.27	**.01**	.29	**.04**
Tibial	126 (37)	41 (31)	56 (33)	.16	.27	.73	.29
Peroneal	21 (6.1)	13 (10)	18 (11)	.33	.10	.66	.25
Dorsalis pedis/pedal	98 (29)	33 (24)	28 (16)	.26	**<.01**	.10	**<.01**
Conduit, No. (%)							
In situ saphenous vein	76 (22)	29 (22)	45 (26)	.92	.30	.36	.53
Reversed saphenous vein	41 (12)	21 (16)	21 (12)	.27	.92	.38	.52
Non-reversed saphenous vein	138 (40)	55 (41)	66 (39)	.84	.70	.63	.88
Arm vein	36 (11)	9 (6.8)	13 (7.7)	.21	.29	.78	.34
Composite vein	19 (5.6)	6 (4.5)	13 (7.7)	.65	.37	.27	.49
Synthetic	42 (12)	16 (12)	20 (12)	.95	.85	.92	.98

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes

details were not significantly different between IDDM and NIDDM patients nor between NIDDM and NDM patients. Single-segment great saphenous vein conduits were used in over three-quarters of procedures performed in each group (76% vs. 80% vs. 78%; P = .56), where non-reversed great saphenous vein was most the most common conduit (40% vs. 41% vs. 39%; P = .88). Composite vein conduit use (6% vs. 5% vs. 8%; P = .49) and synthetic conduit use (12% vs. 12% vs. 12%; P = .98) did not differ between diabetes type.

Finally, of the 648 PTA/S-first procedures, IDDM patients were less likely to undergo a superficial femoral artery angioplasty (57% vs. 67% vs. 75%; P < .001) and were more likely to undergo an anterior tibial angioplasty (31% vs. 11% vs. 16%; P < .001) (Table III). Further, there were no differences in multi-level interventions (42% vs. 42% vs. 49%; P = .34); however, femoropopliteal stenting was significantly less common among IDDM patients (26% vs. 31% vs. 42%; P = .001) – a significant difference that was most likely driven by the difference between IDDM patients and NDM patients (P < .001). NIDDM patients, when compared solely to NDM patients, were significantly less likely to undergo infrapopliteal stenting (3% vs. 9%; P = .045).

The median follow-up for IDDM, NIDDM, and NDM patients was 1.5 years (range <1–10), 1.6 years (<1–10), and 1.3 years (<1–10), respectively.

Table III. Operative details of 648 insulin-dependent, noninsulin-dependent, and non-diabetic patients undergoing percutaneous transluminal angioplasty for chronic limb-threatening ischemia (CLTI)

	IDDM *(N=342)*	**NIDDM** *(N=133)*	**NDM** *(N=171)*	**P-value** (IDDM to NIDDM)	**P-value** (IDDM to NDM)	**P-value** (NIDDM to NDM)	**P-value**
Proximal vessel, No. (%)							
Femoral	204 (57)	86 (67)	118 (75)	**.04**	**<.001**	.14	**<.001**
Popliteal	126 (35)	44 (34)	69 (44)	.87	.06	.10	.13
Infrapopliteal vessel, No. (%)							
Anterior tibial	111 (31)	14 (11)	25 (16)	**<.001**	**<.001**	.22	**<.001**
Posterior tibial	59 (16)	13 (10)	16 (10)	.08	.06	.99	.07
Peroneal	73 (20)	25 (19)	24 (15)	.84	.18	.35	.40
Dorsalis pedis/pedal	10 (3.0)	0 (0.0)	2 (1.3)	.06	.29	.20	.11
Multi-level (prox + infrapop)	152 (42)	54 (42)	77 (49)	.96	.16	.25	.34
Stenting, No. (%)							
Any	109 (30)	43 (33)	71 (45)	.51	**.001**	**.046**	**<.01**
Femoropopliteal	92 (26)	40 (31)	66 (42)	.23	**<.001**	.06	**.001**
Infrapopliteal	26 (7.2)	4 (3.1)	14 (8.9)	.10	.51	**.045**	.14

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes

Perioperative Outcomes

Following any lower extremity revascularization for CLTI, IDDM patients exhibited a significantly longer total hospital length of stay (LOS) (9.6 vs. 8.9 vs. 8.0 days; P < .01), most likely driven by the LOS difference between IDDM and NDM patients (P < .001) (Table IV). Further univariate analysis suggested that both perioperative mortality (3.0 vs. 1.5 vs. 4.9; P = .07) and perioperative complications (15% vs. 12% vs. 15%; P = .60) were similar between groups. Among BPG-first patients, perioperative surgical site infections did not differ (11% vs. 10% vs. 8%; P = .52). Regardless of procedure type, after adjusting for baseline characteristics, multivariable analysis found diabetes type to not be associated with perioperative death or complications.

Table IV. Perioperative outcomes and complications between 1,294 insulin-dependent, noninsulin-dependent, and non-diabetic patients with chronic limb-threatening ischemia (CLTI)

	IDDM *(N=703)*	**NIDDM** *(N=262)*	**NDM** *(N=329)*	**P-value** (IDDM to NIDDM)	**P-value** (IDDM to NDM)	**P-value** (NIDDM to NDM)	**P-value**
Perioperative Outcomes, No. (%)							
Pre-operative LOS, mean days	3.3	3.1	2.4	.39	**<.001**	.05	**<.01**
Post-operative LOS, mean days	6.3	5.8	5.6	.23	.05	.62	.11
Total LOS, mean days	9.6	8.9	8.0	.22	**<.01**	.14	**<.01**
Hematoma	40 (5.7)	15 (5.7)	20 (6.1)	.98	.80	.86	.97
Acute myocardial infarction	13 (1.8)	1 (0.4)	4 (1.2)	.09	.46	.27	.21
Mortality	21 (3.0)	4 (1.5)	16 (4.9)	.20	.13	**.03**	.07

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes, *LOS:* length of stay

Long-term Outcomes

Unadjusted Kaplan-Meier analysis demonstrated that complete wound healing at 6-month follow-up was significantly worse among IDDM patients (36% vs. 40% vs. 51%; P < .001). Further unadjusted Kaplan-Meier analyses illustrated no significant difference in three-year rates of restenosis (50% vs. 46% vs. 38%; P = .36) and re-intervention (36% vs. 37% vs. 31%; P = .63) but did demonstrate significant differences in three-year rates of major amputation (23% vs. 11% vs. 8%; P < .001; Figure 2), RAS events (65% vs. 55% vs. 51%; P = .04; Figure 3), MALE (34% vs. 27% vs. 23%; P < .01; Figure 4), and death (44% vs. 35% vs. 49%; P < .01; Figure 5).

After adjustment, among all procedure types, diabetes type was not shown to independently affect restenosis or re-intervention. Conversely, among all revascularization strategies, with NDM as the reference group, IDDM was shown to independently heighten

a patient's risk of incomplete wound healing (Hazard Ratio (HR) 1.4, 95% Confidence Interval [CI], 1.2-2.9), major amputation (2.4 [1.1-5.1]), RAS events (1.4 [1.1-1.9]) and MALE (1.6 [1.1-2.7]) (Table V). Among BPG-first interventions, IDDM patients were shown to only independently heighten the risk of incomplete wound healing (1.5 [1.3-2.9]). Finally, among PTA/S-first interventions, IDDM patients were shown to independently heighten the risk of incomplete wound healing (1.7 [1.3-3.8]), major amputation (3.5 [1.2-10.6]), and RAS events (1.5 [1.1-2.1]). NIDDM patients, on the other hand, were not shown to be significantly associated with any limb-related primary outcome; however, interestingly, NIDDM, as compared to NDM, was associated with a significantly lower risk of mortality among patients undergoing any revascularization type (0.6 [0.5-0.8]), a BPG-first intervention (0.6 [0.5-0.9]), and a PTA/S-first intervention (0.6 [0.4-0.9]).

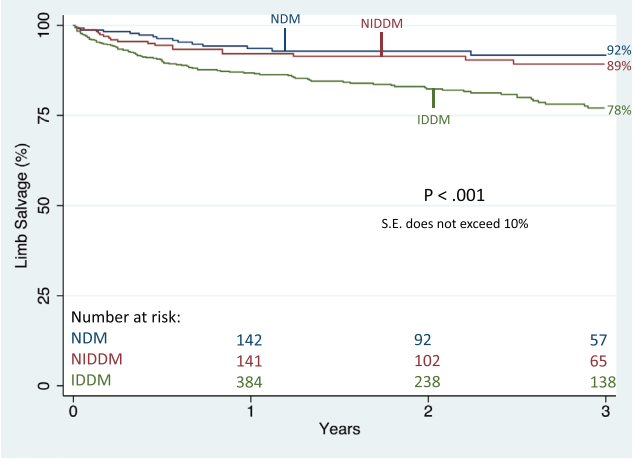

Figure 2. Unadjusted effect of diabetes type on long-term limb salvage among patients undergoing any lower extremity revascularization for chronic limb-threatening ischemia (CLTI)

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes, *S.E.:* standard error

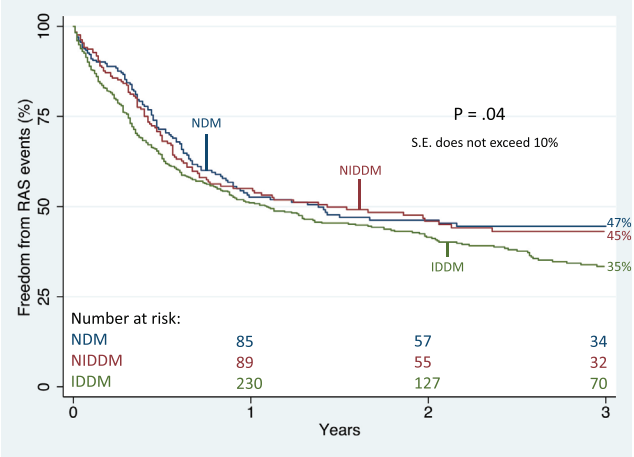

Figure 3. Unadjusted effect of diabetes type on long-term freedom from re-intervention, amputation, or stenosis (RAS) among patients undergoing any lower extremity revascularization for chronic limb-threatening ischemia (CLTI)

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes, *S.E.:* standard error

An important and final note is that, when combining IDDM and NIDDM patients (i.e., comparing 965 diabetic patients vs. 329 non-diabetic patients), multivariable analysis demonstrated that any diabetes was significantly associated with higher risk of incomplete wound healing (1.4, 1.1-1.9]), major amputation (2.4 [1.2-4.8]), and MALE (1.7 [1.1-2.7]), but there was no difference in mortality (0.9 [0.6-1.1]; P = .07).

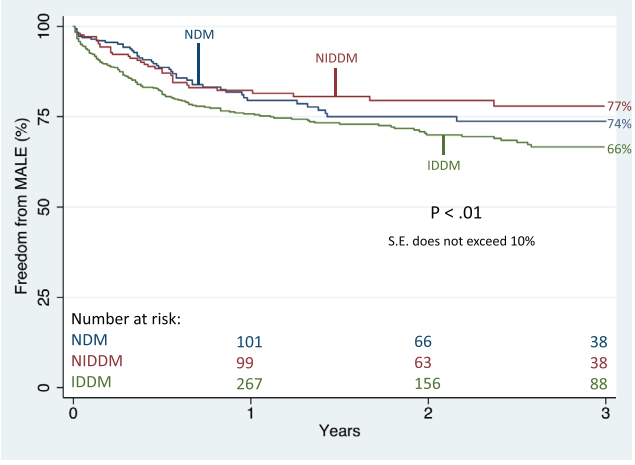

Figure 4. Unadjusted effect of diabetes type on long-term freedom from any major adverse limb event (MALE) among patients undergoing any lower extremity revascularization for chronic limb-threatening ischemia (CLTI)

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes, *S.E.:* standard error

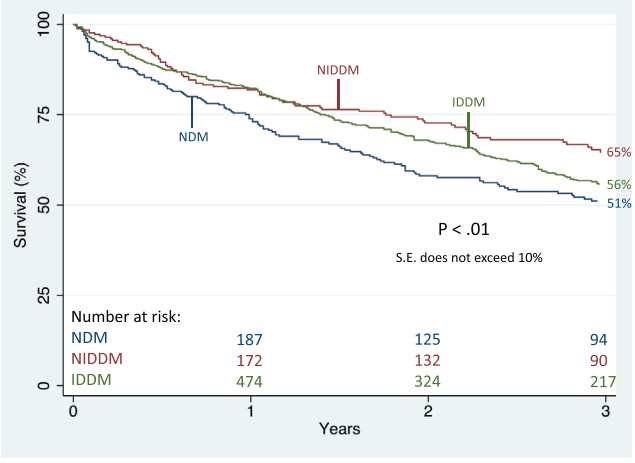

Figure 5. Unadjusted effect of diabetes type on long-term survival among patients undergoing any lower extremity revascularization for chronic limb-threatening ischemia (CLTI)

IDDM: insulin-dependent diabetes, *NIDDM:* noninsulin-dependent diabetes, *NDM*: non-diabetes, *S.E.:* standard error

Table V. Multivariable analyses of diabetes type on long-term major amputation, re-intervention, amputation, or stenosis (RAS) events, and mortality

	Any intervention *(N=1294)*			**Bypass-first** *(N=646)*			**PTA/S-first** *(N=648)*		
Outcomes	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Mortality									
NDM (ref)	-	-	-	-	-	-	-	-	-
NIDDM	0.6	0.5-0.8	**.001**	0.6	0.5-0.9	**.01**	0.6	0.4-0.9	**.01**
IDDM	0.9	0.7-1.1	.29	0.9	0.7-1.3	.65	0.8	0.6-1.2	.28
Major amputation									
NDM (ref)	-	-	-	-	-	-	-	-	-
NIDDM	2.0	0.9-4.9	.23	1.9	0.6-6.2	.28	1.2	0.3-4.5	.76
IDDM	2.4	1.1-5.1	**.01**	2.2	0.8-6.6	.15	3.5	1.2-10.6	**.03**
RAS									
NDM (ref)	-	-	-	-	-	-	-	-	-
NIDDM	0.9	0.6-1.4	.68	1.1	0.6-2.0	.66	0.8	0.5-1.3	.39
IDDM	1.4	1.1-1.9	**.04**	1.5	0.9-2.4	.14	1.5	1.1-2.1	**.03**
MALE									
NDM (ref)	-	-	-	-	-	-	-	-	-
NIDDM	1.1	0.6-1.9	.87	0.9	0.5-1.6	.59	1.0	0.4-2.6	.99
IDDM	2.0	1.1-2.7	**.04**	1.4	0.8-2.3	.20	1.7	0.8-3.6	.16
Incomplete healing									
NDM (ref)	-	-	-	-	-	-	-	-	-
NIDDM	1.2	0.7-1.2	.30	1.3	0.8-1.7	.16	1.4	0.6-1.9	.20
IDDM	1.4	1.2-3.2	**<.01**	1.5	1.3-2.9	**.01**	1.7	1.3-3.8	**.03**

NDM: no diabetes, *NIDDM:* non-insulin-dependent diabetes, *IDDM:* insulin-dependent diabetes, *HR:* hazard ratio, *CI:* confidence interval, *MALE:* major adverse limb event

Additionally adjusted for age, gender, symptom status, ambulatory status, living status, race, renal disease, coronary artery disease, hypertension, hyperlipidemia, history of myocardial infarction, congestive heart failure, TASC classification, smoking history, COPD, and procedure type

DISCUSSIONS

Our data illustrate that, in patients undergoing a first-time lower extremity revascularization for CLTI, those suffering from IDDM present at an earlier age and with more severe disease. Regardless of revascularization strategy, there are no differences in perioperative complications, restenosis, or re-intervention; however, IDDM was associated with longer pre-operative and total hospital lengths of stay, as well as a heightened risk of incomplete wound healing, major amputation, RAS events, and major adverse limb events. Conversely, NIDDM patients – seemingly the least diseased-burden of the three groups – were shown to have lower long-term mortality (compared to NDM), even after adjusting for the discrepancy in comorbidity burden. More specifically, as compared to NDM patients, IDDM patients undergoing a PTA/S-first strategy were shown to have a heightened risk of incomplete wound healing, RAS events, and major amputation. Conversely, IDDM patients undergoing a bypass-first strategy were shown to only be associated with poorer wound healing, suggesting that the oft-referenced adverse outcomes in IDDM patients may be most mitigated following a bypass-first strategy.

Prior studies have illustrated that the impact of diabetes on perioperative outcomes remains controversial, with several studies demonstrating higher risk of perioperative morbidity and mortality among patients with diabetes, whereas others report no added risk in this patient population.[19-22] In 2004, Virkkunen et al. studied 5,709 lower extremity bypasses performed for CLTI and found that patients with diabetes, although not differing in perioperative mortality, demonstrated a higher risk of below-knee amputation (OR, 1.7), cardiac complications (OR, 1.5), and wound infection (OR, 1.3).[19] Conversely, in two prior analyses from our group, Akbari et al. demonstrated reduced in-hospital mortality in patients with diabetes compared to those without (0.9% vs. 4.2%) and reported no difference between the groups in five-year survival or limb salvage, while Hamdan et al. – reporting perioperative and long-term outcomes among 4,052 lower extremity procedures – also found diabetes to be associated with lower perioperative mortality (OR, 0.6) and to decrease five-year survival, although these were unadjusted rates and no multivariable analysis was performed. [22, 23] Importantly, however, these studies did not distinguish between diabetes type, which, as our data illustrate, may play individual and important roles in long-term risk.

Fortunately, several recent studies have elaborated on the potential importance of diabetes type following lower extremity revascularization. In 2007, Hertzer et al. examined a single institution's experience with 600 lower extremity bypasses for PAD and stratified by type of diabetes, finding no difference in perioperative mortality and significantly higher rates of one-year and five-year mortality among NIDDM (1.4 [1.1–1.8]) and IDDM (1.5 [1.2–1.8]) patients.[24] This study also indicated that IDDM is a significant predictor of both short-term and long-term amputation (OR, 2.6 and OR, 1.8, respectively). Additionally, in 2012, Wallaert et al. analyzed the effect of diabetes type on 1,977 infrainguinal bypass patients with CLTI, demonstrating that diabetes type does not significantly affect perioperative mortality rates and that both NIDDM and IDDM were associated with higher

perioperative rates of any major adverse event, a composite variable defined as myocardial infarction, dysrhythmia, congestive heart failure, renal insufficiency, wound infection, and major amputation (OR, 1.4 and OR 1.5, respectively). Both of these studies focus only on patients undergoing bypass, providing little information regarding a prevalent subset of patients who undergo PTA/S procedures.

Lastly, in 2007, Dick et al. performed a prospective cohort study of 426 limbs suffering from both diabetes and CLTI undergoing conservative treatment, endovascular treatment, or surgical treatment.[25] This study demonstrated that one-year clinical success – defined as survival without major amputation or future target extremity revascularization – was significantly better in non-diabetic patients (HR, 0.48), and that, in both diabetic and non-diabetic patients, this success was not influenced by mode of initial revascularization. Further, diabetes was not shown to be significantly associated with higher one-year mortality (P = .064). Ultimately, diabetic patients within this cohort were shown to improve to the same degree as in non-diabetic patients, but only through multiple revascularization procedures and by means of close follow-up and timely repetition of target extremity revascularization.

Overall, our study both differs from and corroborates previous literature. Curiously, NIDDM patients within our study were shown to have lower long-term mortality, which is a novel finding compared to prior works. Generally, we believe that this outcome may be less reflective of the health of NIDDM patients and more reflective of the severity of disease among and between both IDDM and NDM patients, as NIDDM patients were less likely to have tissue loss, coronary artery disease, and congestive heart failure (as compared to IDDM), and decreased proportions of COPD, smoking history, and femoropopliteal TASC D lesions (as compared to NDM). Although a surprising finding, the lower mortality among NIDDM patients may further reflect better – or simpler – long-term medical management, or the potential additional increases in cardiac disease within the IDDM and NDM patients that is not presently captured within this analysis. Importantly, when combining IDDM and NIDDM groups, our study substantiates the insignificant differences in long-term mortality that several previous studies have demonstrated, further highlighting the importance of evaluating the distinction between insulin-dependent and noninsulin-dependent diabetes within CLTI patients.[21, 22, 24, 25]

There are important limitations to this study. First, it was a retrospective, single-center review where patients were allocated to treatment based on surgeon preference, which changed over time. As our data represent the experience of one group of surgeons at a single institution, the potential for selection and information bias exists and our results are subject to the influence of specific referral patterns, surgeon experience, and patient selection preferences. Second, these data only include revascularization attempts and do not reflect outcomes for those patients treated with primary amputation or medical management as a contrast. Fortunately, several previously published studies have illustrated both the poor outcomes following medical management and the importance of revascularization in diabetic patients with CLTI.[20, 21, 25-28] Lastly, since supplementary

measures of diabetes disease severity were not readily accessible for this study, including patient hemoglobin A1c, baseline insulin reliance and administration was used as a replacement for disease severity, which could increase potential for confounding factors. Ultimately, however, our data include one of the largest reported analyses of the effect of diabetes type on the initial revascularization for CLTI.

CONCLUSIONS

To conclude, our data suggest that insulin-dependent patients undergoing any first-time revascularization for CLTI may have a disease severity-dependent limb effect on a variety of long-term outcomes. Noninsulin dependence is not associated with these long-term events and, as compared to non-diabetic patients, is actually associated with lower long-term mortality. Overall, these data demonstrate the importance in distinguishing between diabetes type, as insulin-dependent, noninsulin-dependent, and non-diabetic patients all present with differing degrees of disease and comorbid conditions that harbor varying degrees of limb-based and patient-based risk. Finally, although insulin-dependence is associated with the greatest risk of adverse outcomes, these data suggest that these adversities may be most mitigated in those IDDM patients that are appropriately selected and anatomically suitable for a bypass.

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CHAPTER 4

LONG-TERM MORTALITY BENEFIT OF RENIN ANGIOTENSIN SYSTEM INHIBITORS IN CHRONIC LIMB-THREATENING ISCHEMIA PATIENTS UNDERGOING VASCULAR INTERVENTION

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ABSTRACT

Objective

The beneficial effect of renin-angiotensin system (RAS) inhibitors has been well established in patients with cardiovascular disease; however, their effectiveness in patients with chronic limb-threatening ischemia (CLTI), a selected disease-burdened population, is largely unknown. The purpose of this study was to evaluate long-term outcomes of RAS inhibitor use in patients with CLTI undergoing a vascular intervention.

Methods

For this study, all CLTI patients undergoing a first-time revascularization (bypass or endovascular) were analyzed at our institution between 2005 and 2014. Patients discharged on a RAS inhibitor (angiotensin-converting enzyme [ACE] inhibitor or angiotensin receptor [AR] blocker) were compared to those not on a RAS inhibitor. Inverse probability of treatment weighting with additional regression analyses were used to determine long-term risk of mortality and major adverse events. A sensitivity analysis was performed to assess the dose-related therapeutic response of RAS inhibitors (low-dose vs. high-dose therapy).

Results

Between 2005 and 2014, 1,303 limbs from 1,161 patients were identified. Of these patients, 52% were discharged on a RAS inhibitor, with 67% discharged on a high-dose therapy and 33% on a low-dose therapy. Patients discharged on a RAS inhibitor suffered more frequently from diabetes, hypertension, and myocardial infarction, whereas those not on a RAS inhibitor had more chronic kidney disease (all $P<.05$). There was no difference in the proportion of patients presenting with tissue loss. After adjustment for these and other baseline covariates, RAS inhibitor use was associated with lower late mortality (hazard ratio [HR] 0.78, [95% confidence interval] 0.65-0.94). Discharge on a high-dose RAS inhibitor was associated with lower mortality (HR 0.70 [0.57-0.86]), while a low-dose RAS inhibitor was not associated with lower mortality (HR 0.95 [0.73-1.24]) compared to patients not prescribed a RAS inhibitor. This association was still significant comparing high-dose to low-dose therapy (HR 0.74 [0.55-0.98]). No associations were found between RAS inhibitor use and major adverse limb event (HR 0.95 [0.73-1.22]), major amputation (HR 0.82 [0.57-1.18]), or reintervention (HR 1.05 [0.85-1.31]). These point estimates were not different for those on ACE inhibitors versus AR blockers, nor were they affected by the type of revascularization.

Conclusions

CLTI patients prescribed a RAS inhibitor at discharge demonstrated significantly lower long-term mortality, whereas limb events were unaffected. These data indicate that, in these heavily burdened patients, the benefit is restricted to those on a high-dose, which underscores the importance of attaining these doses.

INTRODUCTION

Physician adherence to guideline-recommended medical management for patients with peripheral arterial disease (PAD) is low, with multiple studies indicating that PAD patients are consistently undertreated.[1-6] While several medications, including statins and antiplatelet agents, have emerged as standard therapies for mitigating surgical adverse events, renin-angiotensin system (RAS) inhibitors tend to be relatively underutilized. Particularly among patients with chronic limb-threatening ischemia (CLTI), aggressive risk factor modification poses a major opportunity to improve cardiac outcomes and survival.

Blockade of RAS by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor (AR) blockers is currently a moderate recommendation (class IIa) in the 2016 lower extremity PAD practice guidelines from the American College of Cardiology and American Heart Association.[7] These recommendations are primarily based on well-designed cardiovascular trials showing major long-term benefit of RAS inhibitors.[8-10] Aside from a few studies reporting outcomes in symptomatic and asymptomatic PAD patients treated with RAS inhibitors,[6,11-14] there is limited evidence specifically in patients with CLTI. In a single study evaluating CLTI patients undergoing diagnostic angiography or endovascular intervention, Armstrong et al. determined that ACE inhibitor or AR blocker prescription was associated with significantly lower mortality and cardiac complications.[15] It remains unclear, however, whether these favorable outcomes demonstrated in endovascular treatment modalities are different for those treated with bypass and, in particular, whether these effects are being realized in a dose-dependent manner. Despite recommendations in cardiovascular practice guidelines to achieve elevated target doses, patients often receive doses that are lower than used in the benchmark clinical trials.[16,17]

Therefore, with this study, we sought to evaluate long-term outcomes of RAS inhibitor use in CLTI patients undergoing a first-time vascular intervention, and determine whether these outcomes are different between low-dose and high-dose therapies.

METHODS

This was a retrospective chart review using data from a single institution between 2005 and 2014. All patients with CLTI undergoing a first-time infrainguinal vascular intervention (endovascular or surgical) were identified through a detailed review of procedure bookings, institutional Vascular Study Group of New England (VSGNE) data, and billing data. The study protocol was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center and a waiver of informed consent was granted due to the retrospective nature of the study.

RAS inhibitor

Medical management was determined in a standardized fashion by individually reviewing preprocedural and discharge medications. To ascertain longer use of RAS inhibitors, we defined our main exposure variable as discharge on an ACE inhibitor or AR blocker. Since we evaluated the effect of discharge medication, we excluded patients who died during their index hospitalization (N = 14; 1.1%). In a separate analysis, we classified those on RAS inhibitors according to their dose intensity into low-dose or high-dose therapy (Table I; presented as daily dose). Because there are no specific recommendations in place regarding appropriate dosing regimens, cutoff values were chosen based on medical and surgical guidelines, randomized controlled trails, and observational studies.[17-20]

Table I. Treatment doses of drugs included in the study cohort

	Low-dose RAS inhibitor *(N = 221)*			**High-dose RAS inhibitor** *(N = 454)*		
	Daily dose	*N*	*%*	*Daily dose*	*N*	*%*
ACE inhibitor						
Lisinopril	≤ 5 mg	116	(52)	10-80 mg	305	(67)
Enalapril	≤ 5 mg	7	(3.2)	10-40 mg	23	(5.1)
Captopril	≤ 50 mg	12	(5.4)	75-300 mg	3	(0.7)
Ramipril	≤ 2.5 mg	4	(1.8)	5-20 mg	9	(2.0)
Quinapril	≤ 20 mg	4	(1.8)	> 20 mg	4	(0.9)
Fosinopril	≤ 20 mg	0	(0)	> 20 mg	2	(0.4)
Moexipril	≤ 7.5 mg	0	(0)	15-30 mg	4	(0.9)
Benazepril	≤ 10 mg	0	(0)	> 10 mg	1	(0.2)
AR blocker						
Losartan	≤ 50 mg	40	(18)	75-200 mg	25	(5.5)
Valsartan	≤ 80 mg	29	(13)	120-320 mg	44	(9.7)
Irbesartan	≤ 150 mg	6	(2.7)	300 mg	7	(1.5)
Olmesartan	≤ 20 mg	2	(0.9)	> 20 mg	2	(0.4)
Candesartan	≤ 8 mg	0	(0)	16-32 mg	5	(1.1)
Telmisartan	≤ 40 mg	1	(0.5)	> 40 mg	0	(0)
Dual ACE or dual AR					3	(0.7)
Combination ACE and AR					17	(3.7)

RAS: renin-angiotensin system, *ACE*: angiotensin-converting enzyme, *AR*: angiotensin receptor

Patients discharged on two RAS inhibitors were automatically designated to the high-dose group (N = 20; 1.5%). All other baseline variables were obtained through clinical notes, admission history, documentation during hospitalization, operative notes, and discharge summaries.

Outcomes

The primary outcome of this study was death throughout follow-up. Death was captured through patient chart review and the Social Security Death Index. Secondary outcomes included 30-day wound infection and a 30-day composite outcome of myocardial infarction or postdischarge mortality, as well as late limb events any time during follow-up, such as major adverse limb event (MALE), major amputation, and reintervention. Wound infection was considered when any surgical site infection was documented. MALE was defined as an ipsilateral major amputation (above or below knee) or major surgical revision, such as new bypass graft, jump or interposition bypass revision, thrombectomy, and pharmacologic or mechanical thrombolysis.[21] Reintervention included any ipsilateral surgical or endovascular revision. Limb events were ascertained through post-procedural clinical visits. Typical follow-up with a vascular surgeon consisted of outpatient care every 3 to 4 months for the first 2 years and every 6 months for the years thereafter. In an attempt to account for repeated measures in patients operated on both limbs, we censored the initial limb at the procedure date of the contralateral limb for our survival analysis, whereas these were not censored when we evaluated limb events.

Statistical analysis

In our primary analysis, patients discharged on any RAS inhibitor were compared to those not discharged on a RAS inhibitor. Next, we determined whether these associations varied for those patients on different dose intensities (low-dose vs. high-dose therapy). In an additional sensitivity analysis, by incorporating interaction terms, we assessed whether the associations found were modified by the type of RAS inhibitor (ACE inhibitor vs. AR blocker), coronary artery disease, or revascularization strategy. Categorical variables were evaluated with the Pearson's χ^2 and Fisher's exact test, and continuous variables were analyzed with the Student's t-test and Mann Whitney U test, where appropriate. Inverse probability of treatment weighting (IPTW) using propensity scores was performed in an attempt to reduce potential confounding and eliminate the bias of nonrandom treatment assignment. Covariates were generously introduced to construct the propensity score and included: age, gender, index procedure (bypass or endovascular), year of procedure, symptom status (rest pain, ulcer, gangrene), race, smoking (prior and current), chronic obstructive pulmonary disease, hypertension, coronary artery disease, history of myocardial infarction, history of coronary intervention, chronic heart failure, hyperlipidemia, diabetes, chronic kidney disease, and discharge on statin medications. Balance diagnostics were examined to verify adequate overlap of propensity scores between treatment groups and included visual inspection of the kernel density plot and assessment of standardized differences and variance ratios.

Doubly robust estimation was used, which combines Cox proportional hazard models with IPTW, to additionally adjust for residual confounding and to further increase the precision of the estimates.[22] These confounders were determined using purposeful selection combining covariates selected a priori and by univariate screen ($P < .10$).[23] We evaluated the proportional hazards assumptions with the scaled Schoenfeld residuals on time and visually by log-log plots. Exact logistic regression was performed to evaluate 30-day outcomes, as an alternative for the maximum likelihood estimation of conventional logistic regression, which appropriately accounts for the rarity of the adverse events. Crude survival between treatment groups was compared using the log-rank test, and adjusted survival curves were constructed after IPTW of the total cohort. All analyses were conducted with SPSS Statistics 23 (IBM Corp, Armonk, NY) and STATA 14 (StataCorp, College Station, Tex).

RESULTS

A total of 1,303 limbs from 1,161 patients were included. Over the study period, 52% of patients with CLTI were discharged on a RAS inhibitor and of those patients 67% were discharged on a high-dose therapy and 33% on a low-dose therapy. Between 2005 and 2014, the proportion of patients who were discharged on a RAS inhibitor within our institution varied but gradually increased, from 53% to 66% after a first-time vascular intervention (Figure 1). This increase was evident among patients discharged on both a low-dose (19% to 27%) and a high-dose therapy (34% to 39%).

Baseline characteristics

As detailed in Table II, demographics were comparable between patients discharged on a RAS inhibitor to those not discharged on a RAS inhibitor. In terms of comorbidities, CLTI patients discharged on a RAS inhibitor were more likely to have diabetes, hypertension, history of myocardial infarction, and hyperlipidemia, while they less frequently had a history of chronic kidney disease and dialysis dependence (all $P < .05$). Patients receiving RAS inhibitors had similar rates of other discharge medications compared to those who did not receive a RAS inhibitor, including antiplatelet agents, statins, and beta-blockers. Finally, independent ambulation was more common in patients discharged on a RAS inhibitor. Univariate outcomes stratified by dose intensity are presented in Supplementary Table I.

Univariate outcomes

Any RAS inhibitor vs. no RAS inhibitor

The median follow-up was 1.5 years (Interquartile range [IQR]: 0.4 – 3.8). The unadjusted survival rates for patients discharged on a RAS inhibitor were significantly higher, with 85% alive at one year, 67% at three years, and 46% at five years, compared to 80%, 54%, and 39% for those not discharged on a RAS inhibitor (log-rank: $P < .001$, standard error [SE] < .10). The incidence of five-year MALE (22% vs. 20%, $P = .50$), major amputation (11% vs.

Table II. Baseline characteristics of patients with and without a renin-angiotensin system (RAS) inhibitor at discharge

	No RAS inhibitor *(N = 628; 48%)*		**Any RAS inhibitor** *(N = 675; 52%)*		
	N	*%*	*N*	*%*	P-value
Age (years, mean ± SD)	71.6	(13)	71.3	(12)	.64
Male gender	389	(62)	384	(57)	.06
Bypass (vs. Endovascular)	313	(50)	341	(51)	.81
Race					.08
White	502	(80)	515	(77)	
Black	63	(10)	95	(14)	
Other	60	(9.6)	63	(9.4)	
Smoking history	375	(61)	406	(61)	.84
Current smoker	129	(21)	143	(22)	.75
Symptom status					.16
Rest pain	127	(20)	125	(19)	
Ulcer	312	(50)	371	(55)	
Gangrene	189	(30)	179	(27)	
Diabetes mellitus	429	(69)	534	(80)	**<.001**
Coronary artery disease	305	(50)	352	(53)	.24
History of myocardial infarction	145	(24)	189	(29)	**.04**
Atrial fibrillation	122	(22)	110	(18)	.07
History of CABG/PCI	217	(35)	239	(36)	.74
Congestive heart failure	192	(31)	206	(31)	.98
Hypertension	499	(81)	600	(90)	**<.001**
Hyperlipidemia	357	(58)	425	(64)	**.03**
Chronic kidney disease	241	(39)	183	(28)	**<.001**
Dialysis dependence	164	(27)	93	(14)	**<.001**
COPD	79	(13)	75	(11)	.41
CVA	75	(13)	86	(14)	.86
Preoperative medication					
Antiplatelet	381	(64)	445	(69)	.06
Lipid-lowering agents	373	(60)	477	(73)	**<.001**
Beta-blocker	360	(60)	391	(60)	.79
Discharge medication					
Antiplatelet	561	(91)	616	(93)	.16
Lipid-lowering agents	474	(76)	540	(80)	.07
Beta-blocker	460	(74)	494	(74)	>.99
Ambulatory independent	313	(54)	383	(61)	**.02**

SD: standard deviation, *CABG*: coronary artery bypass grafting, *PCI*: percutaneous coronary intervention, *COPD*: chronic obstructive pulmonary disease, *CVA*: cerebrovascular accident

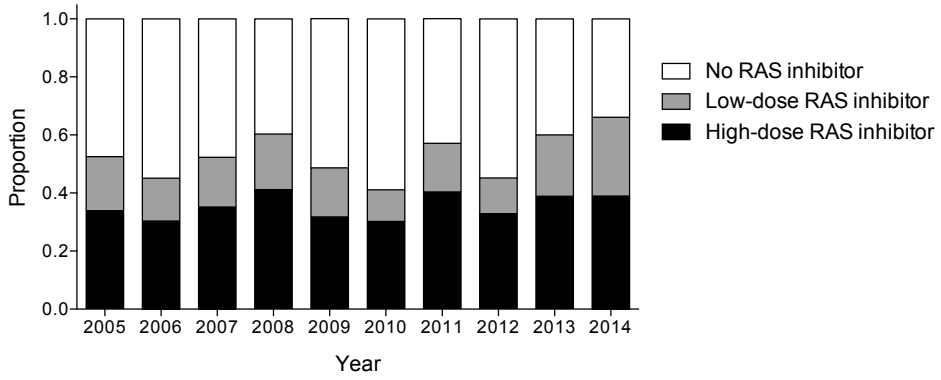


Figure 1. Proportions of patients on various renin-angiotensin system (RAS) inhibitor intensities by year

11%, P = .60), and reintervention (31% vs. 27%, P = .11) did not significantly differ between those receiving and not receiving a RAS inhibitor. On univariate analysis, 30-day myocardial infarction or post-discharge mortality was lower among patients discharged on a RAS inhibitor compared to those who were not (1.6% vs. 4.3%, P < .01). Myocardial infarction occurred less often in those prescribed a RAS inhibitor (0.4% vs. 1.9%, P = .01), while post-discharge mortality was not significantly different (1.2% vs. 2.5%, P = .07).

Table III. Univariate outcomes of patients on various renin-angiotensin system (RAS) inhibitor intensities

	No RAS inhibitor		**Low-dose RAS inhibitor**		**High-dose RAS inhibitor**		**P-value**	
	(N = 628; 48%)		*(N = 221; 17%)*		*(N = 454; 35%)*		Overall	Low- vs. high-dose
	N	*%*	*N*	*%*	*N*	*%*		
30-day outcomes								
Mortality or myocardial infarction	27	(4.3)	5	(2.3)	6	(1.3)	**.01**	.37
Mortality	16	(2.5)	4	(1.8)	4	(0.9)	.13	.45
Myocardial infarction	12	(1.9)	1	(0.5)	2	(0.4)	**.046**	>.99
Wound infection	39	(6.2)	10	(4.5)	22	(4.8)	.50	.85
Long-term outcomes								
MALE	128	(20)	49	(22)	99	(22)	.79	.91
Major amputation	66	(11)	22	(10)	55	(12)	.61	.41
Reintervention	171	(27)	68	(31)	143	(32)	.27	.88

MALE: major adverse limb event

RAS inhibitor dose intensity

Survival was higher in those discharged on high-dose compared to low-dose RAS at one year (89% vs. 79%), three years (71% vs. 59%), and five years (50% vs. 39%) (log-rank P < .01, SE < .10). Limb events through five years were comparable between patients discharged on different dose intensities (Table III). There was no difference in 30-day myocardial infarction or post-discharge mortality between patients on a high-dose versus low-dose RAS inhibitor (P = .37).

Multivariable analysis

Any RAS inhibitor vs. no RAS inhibitor

Following inverse probability of treatment weighting with additional adjustment for key confounders (e.g., age, symptom status, cardiovascular disease, and chronic kidney disease), RAS inhibitor use was associated with a 22% lower rate of death (hazard ratio [HR] 0.78, 95% confidence interval [CI] 0.65 – 0.94) (Figure 2 A). No significant associations were found regarding RAS inhibitor use and MALE (HR 0.95, 95% CI 0.73 – 1.22), major amputation (HR 0.82, 95% CI 0.57 – 1.18), and reintervention (HR 1.05, 95% CI 0.85 – 1.31) (Table IV). After adjustment, RAS inhibitors were independently associated with reduced incidence of 30-day myocardial infarction or post-discharge mortality (odds ratio [OR] 0.41, 95% CI 0.18 – 0.88) (Figure 3).

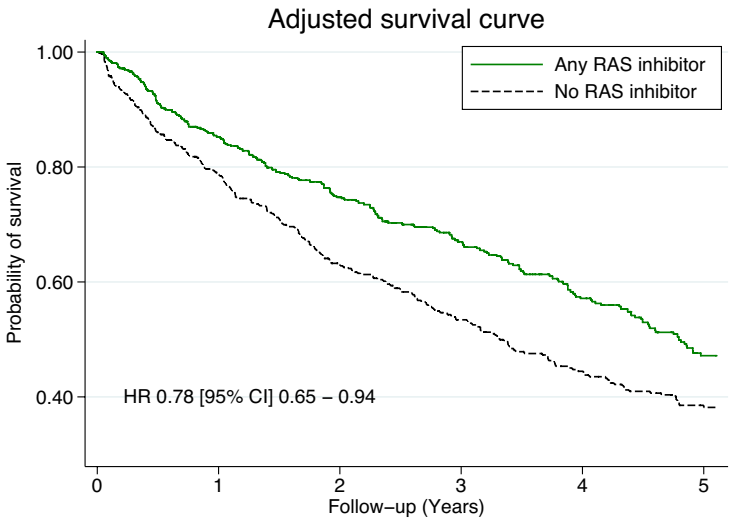
A sensitivity analysis showed similar point estimates between patients prescribed ACE inhibitors versus AR blockers. In addition, survival associated with RAS inhibitor use was not modified by a history of coronary artery disease or the type of procedure (surgical vs. endovascular) (interaction: P > .05).

Table IV. Adjusted associations between various renin-angiotensin system (RAS) inhibitor intensities and adverse outcomes

	Any RAS inhibitor			**Low-dose RAS inhibitor**			**High-dose RAS inhibitor**		
5-year limb events	*HR*	*[95% CI]*	*P-value*	*HR*	*[95% CI]*	*P-value*	*HR*	*[95% CI]*	*P-value*
MALE	0.95	[0.73 – 1.22]	.68	1.00	[0.71 – 1.43]	.98	0.93	[0.70 – 1.23]	.61
Major amputation	0.82	[0.57 – 1.18]	.29	0.72	[0.43 – 1.22]	.23	0.87	[0.59 – 1.30]	.50
Reintervention	1.05	[0.85 – 1.31]	.63	1.16	[0.87 – 1.56]	.31	1.01	[0.79 – 1.28]	.95

HR: hazard ratio, *CI*: confidence interval, *MALE*: major adverse limb event
Additionally adjusted for: age, gender, race, symptom status, diabetes, coronary artery disease, hypertension, history of myocardial infarction, congestive heart failure, COPD, chronic kidney disease, procedure, procedure year, discharged on statin

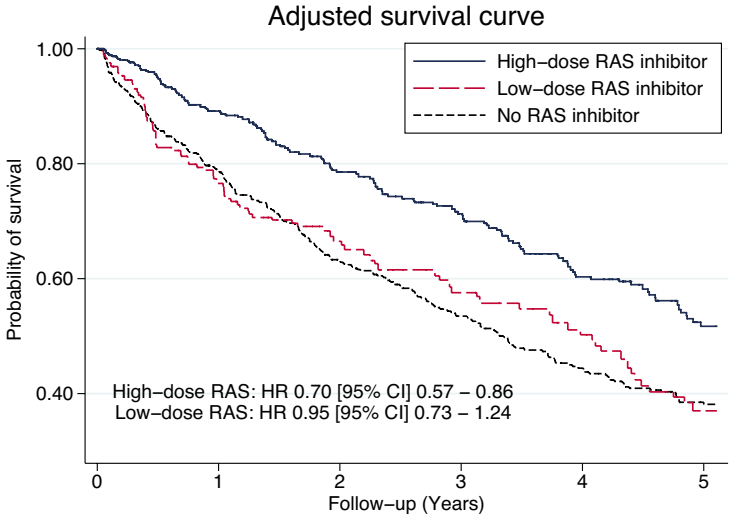
A.



Number at risk

Any RAS inhibitor	675	442	325	250	176	117
No RAS inhibitor	628	380	268	199	146	104

B.



Number at risk

High-dose RAS inhibitor	454	312	233	180	146	83
Low-dose RAS inhibitor	221	130	92	70	51	34
No RAS inhibitor	628	380	268	199	146	104

Figure 2. Adjusted survival curves of patients on various renin-angiotensin system (RAS) inhibitor intensities **A.** Any RAS inhibitor vs. no RAS inhibitor **B.** RAS inhibitor dose intensity (with no RAS inhibitor as the reference group)

RAS inhibitor dose intensity

To evaluate the dose-related therapeutic response of RAS inhibitors, outcomes were compared between dose intensities in an adjusted analysis after inverse probability of treatment weighting. Treatment with a high-dose RAS inhibitor was significantly associated with 30% lower mortality (HR 0.70, 95% CI 0.57 – 0.86), while we found no association between late survival and treatment with a low-dose RAS inhibitor (HR 0.95, 95% CI 0.73 – 1.24) compared to no RAS inhibitor (Figure 2 B). The association found was still significant comparing high-dose to low-dose therapy (HR 0.74 [0.55-0.98]). Similar to the primary analysis, there were no notable associations between either RAS inhibitor dose group and patients without a RAS inhibitor in respect to MALE, major amputation, or reintervention (Table IV). Patients on a RAS inhibitor at a high-dose demonstrated a 65% lower rate of the composite endpoint, 30-day myocardial infarction or post-discharge mortality (OR 0.35, 95% CI 0.11 – 0.88), whereas this association was not significant in those on low-dose therapy (OR 0.53, 95% CI 0.16 – 1.45) (Figure 3).

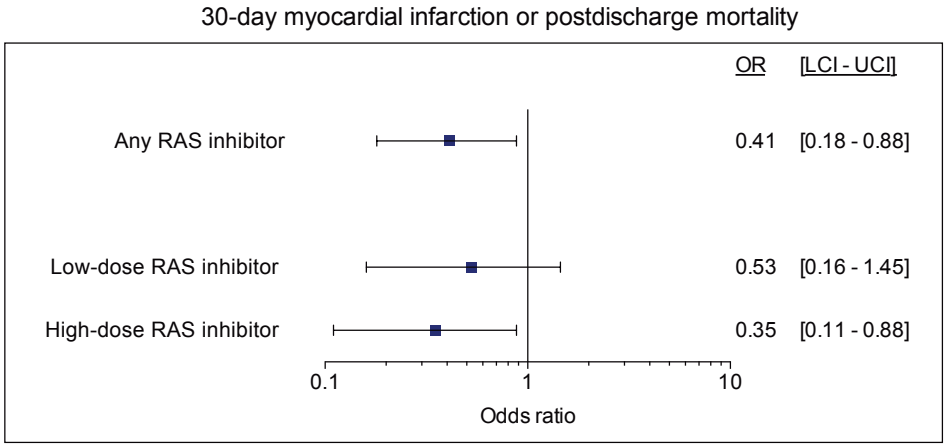


Figure 3. Adjusted associations between renin-angiotensin system (RAS) inhibitors and 30-day myocardial infarction or postdischarge mortality, with no RAS inhibitor as the reference group

OR: odds ratio, *LCI*: lower confidence interval, *UCI*: upper confidence interval. Confidence intervals are 95%. Adjusted for: age, gender, symptom status, coronary artery disease

DISCUSSION

This study demonstrates that CLTI patients prescribed RAS inhibitors at discharge had significantly lower periprocedural cardiac events or death, as well as improved survival up to five years after a first-time revascularization. There was no significant benefit of RAS inhibitors in regard to adverse limb events, including MALE and major amputation. Patients prescribed a high-dose therapy experienced a lower mortality (reduction of 30%), whereas those on a low-dose RAS inhibitor did not demonstrate any survival or limb-related benefits over those not discharged on a RAS inhibitor.

Smoking cessation, weight loss, and exercise programs are common secondary prevention measures in PAD patients, as is the prescription of some form of antiplatelet or statin. Adherence to secondary prevention strategies seems to improve over time, however, only about half of patients are prescribed a RAS inhibitor, ranging from 20% to 60% in the current literature, despite compelling evidence of their known cardiovascular benefit.[1-6] In a selected subset of patients from the multicenter HOPE trial, Östergren et al. reported that ramipril treatment (10 mg/day) in 1,725 symptomatic PAD patients was associated with a 25% relative risk reduction of cardiovascular death, a non-significant 15% lower rate of all-cause mortality, and a 25% myocardial infarction risk reduction.[11] It is interesting to note that these findings could only be partly attributed to a blood pressure lowering effect, since there was only a modest decrease in mean blood pressure (systolic of 3 mmHg and diastolic of 2 mmHg). Observational studies have shown similar results. With a median follow-up of eight years in 2,420 PAD patients (defined as ankle-brachial index ≤0.9), Feringa et al. determined that not only statins, antiplatelets, and beta-blockers were independently associated with lower mortality but also ACE inhibitors (HR 0.80, 95% CI 0.69 – 0.94).[6]

The above-mentioned studies clearly show a benefit in patients with atherosclerotic disease of the lower extremities; however, limited studies specifically address the most severe manifestation of PAD: CLTI. One other single-institution study was conducted among 464 CLTI patients undergoing diagnostic angiography or endovascular intervention using a similar propensity weighted analysis. Although not stratified by dose intensity, this study did demonstrate a significantly lower risk of major adverse cardiovascular event (HR 0.76, 95% CI 0.58 – 0.99) and mortality (HR 0.71, 95% CI 0.53 – 0.95) at three years in those prescribed either an ACE inhibitor or AR blocker.[15] This highlights the value of best medical management and, ultimately, prescription of RAS inhibitors should be considered in all CLTI patients, as this is a particularly vulnerable population that seems to benefit from optimization of cardiovascular health. Similar to the current study, the prior report failed to demonstrate a limb-specific benefit in those with more advanced disease on RAS inhibitors. However, this does not necessarily imply that there is no effect on lower extremity vasculature. Among patients with claudication, it has been suggested that RAS inhibitors may reduce symptoms and improve functional capacity, such as walking distance and pain-free walking time.[12-14] This study did not specifically investigate these parameters and,

therefore, further data are needed to explore whether RAS inhibitors could play a role in improving these quality of life measures in the CLTI population.

Our data extend previous findings and support the idea of titration to higher doses of RAS inhibitors to achieve better clinical outcomes in patients with CLTI, unless limited by side effects. This is reflected by numerous clinical trials in primarily patients with congestive heart failure.[16,20,24,25] Prescription of low-dose therapy may be based upon the belief that high and low doses exert similar benefits but without the side effects of high doses. Yet, our study, together with multiple others, indicates that high doses result in improved clinical outcomes.[20,24] Although not assessed in the present analysis, additional toxicity of high doses is only infrequently documented (approximately less than 5%) and this may be instigating overly cautious reluctance among physicians to continue RAS inhibitors after only a modest decrease in blood pressure or after a decline in renal function. It is a misconception that patients with chronic kidney disease cannot be treated with RAS inhibitors. While close monitoring is recommended, particularly among patients with renal artery stenosis, RAS inhibition is associated with reducing proteinuria and slowing the rate of progression of kidney disease.[26-28] In the occurrence of acute renal failure, which most commonly develops shortly after initiation, RAS inhibitors can easily be discontinued whereupon renal deterioration is reversible. These reservations may have limited the widespread utilization and appropriate dosing of RAS inhibitors, while the advantages grossly outweigh the potential risks. Since low-dose therapy did not prolong life expectancy in CLTI patients, low dosing regimens that are now widely used in clinical practices may deprive these patients from the potential benefit of RAS inhibitors.

These data further suggest that the cardiovascular protective effects illustrated in this study were not different among patients discharged on ACE inhibitors or AR blockers. The proportion of patients prescribed an AR blocker was smaller, and thus we may have failed to detect a difference due to lack of power. However, our results are consistent with a prior meta-analysis that showed, in a pooled estimate of 49,924 patients, equal benefits between both RAS-based medications in respect to myocardial infarction, cardiovascular mortality, and overall mortality.[29] In addition, the survival benefit of RAS inhibitors did not vary across the type of procedure (surgical or endovascular) or among patients with and without coronary artery disease. Therefore, these data support the implementation of guideline-recommended prescription of RAS inhibitors in the vast majority of patients with CLTI.

Several limitations warrant discussion. Firstly, our study was subject to the general limitations inherent to observational studies, and although we have attempted to account for this with propensity-adjusted analyses, the possibility of residual and unmeasured confounding still exists. Secondly, caution must be taken when generalizing these findings beyond the boundaries of our institution. Moreover, the main exposure variable was based on discharge medications and, unfortunately, we lack specific data on compliance and change in doses over time. Understanding the reason for non-adherence to RAS inhibitors would have added extra detail, however, side effects were not documented within our registry, such as hypotension, hyperkalemia, and renal deterioration. In addition, direct

renin inhibitors, the most recent class of RAS inhibitors, were not assessed in the present study. Finally, patients treated with primary amputation or medical management only were not identified in the current cohort and, although this is a relatively small subgroup, it may compromise the study's generalizability to the entire CLTI population.

CONCLUSION

This study demonstrates that RAS inhibitor use in CLTI patients was associated with lower cardiovascular adverse events and improved long-term survival, although this benefit was only observed in those on a high-dose therapy. Therefore, physicians should strive to maintain patients on a high-dose RAS inhibitor, provided that such doses are tolerated. It is likely that a change in current prescribing patterns would benefit a large number of patients with CLTI, which provides an opportunity for physicians to not only optimize surgical care but also medical management.

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SUPPLEMENTARY

Supplementary table I. Baseline characteristics of patients on various renin-angiotensin system (RAS) inhibitor intensities

	No RAS inhibitor		**Low-dose RAS inhibitor**		**High-dose RAS inhibitor**		**P-value**	
	(N = 635; 48%)		*(N = 224; 17%)*		*(N = 458; 35%)*		Overall	Low- vs. High-dose
	N	*%*	*N*	*%*	*N*	*%*		
Age (years, mean ± SD)	71.6	(13)	72.0	(12)	70.9	(12)	.51	.26
Male gender	389	(62)	132	(60)	252	(56)	.10	.30
Bypass (vs. endovascular)	313	(50)	106	(48)	235	(52)	.63	.35
Race							**.001**	**<.01**
White	502	(80)	185	(85)	330	(73)		
Black	63	(10)	18	(8.2)	77	(17)		
Other	60	(9.6)	16	(7.3)	47	(10)		
Smoking history	375	(61)	146	(67)	260	(58)	.11	**.04**
Current smoker	129	(21)	51	(23)	92	(21)	.70	.44
Symptom status							.36	.69
Rest pain	127	(20)	37	(17)	88	(19)		
Ulcer	312	(50)	123	(56)	248	(55)		
Gangrene	189	(30)	61	(28)	118	(26)		
Diabetes mellitus	429	(69)	174	(80)	360	(80)	**<.001**	.99
Coronary artery disease	305	(50)	130	(60)	222	(49)	**.02**	**.01**
History of myocardial infarction	145	(24)	81	(38)	108	(24)	**<.001**	**<.001**
Atrial fibrillation	122	(22)	49	(24)	61	(15)	**<.01**	**<.01**
History of CABG/PCI	217	(35)	92	(43)	147	(33)	**.045**	**.01**
Congestive heart failure	192	(31)	78	(36)	128	(29)	.15	.05
Hypertension	499	(81)	186	(85)	414	(92)	**<.001**	**.01**
Hyperlipidemia	357	(58)	137	(63)	288	(64)	.08	.72
Chronic kidney disease	241	(39)	62	(29)	121	(27)	**<.001**	.65
Dialysis dependence	164	(27)	37	(17)	56	(13)	**<.001**	.12
COPD	79	(13)	29	(13)	46	(10)	.39	.25
CVA	75	(13)	27	(13)	59	(14)	.94	.77
Preoperative medication								
Antiplatelet	381	(64)	143	(69)	302	(69)	.17	.99
Lipid-lowering agents	373	(60)	161	(75)	316	(71)	**<.001**	.27
Beta-blocker	360	(60)	132	(64)	259	(59)	.50	.25
Preoperative medication								
Antiplatelet	561	(91)	204	(95)	412	(92)	.12	.11
Lipid-lowering agents	474	(76)	180	(82)	360	(80)	.15	.47
Beta-blocker	460	(74)	160	(74)	334	(74)	>.99	.97
Ambulatory independent	313	(54)	112	(54)	271	(64)	**<.01**	**.01**

SD: standard deviation, *CABG*: coronary artery bypass grafting, *PCI*: percutaneous coronary intervention, *COPD*: chronic obstructive pulmonary disease, *CVA*: cerebrovascular accident

CHAPTER 5

PREOPERATIVE ANEMIA ASSOCIATED WITH ADVERSE OUTCOMES AFTER INFRAINGUINAL BYPASS SURGERY IN PATIENTS WITH CHRONIC LIMB-THREATENING ISCHEMIA

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ABSTRACT

Objective

Preoperative anemia in elderly patients undergoing surgery is prevalent and associated with adverse events; however, the interaction with other risk factors in patients with chronic limb-threatening ischemia (CLTI) is not well described. The purpose of this study was to assess the association between lower hematocrit (HCT) on admission and postoperative outcomes after infrainguinal bypass surgery.

Methods

Patients with CLTI undergoing non-emergent infrainguinal bypass were identified in the targeted vascular NSQIP 2011-2014. Thirty-day outcomes were compared across preoperative HCT levels: severe (HCT ≤29%), moderate (HCT 29.1-34%), mild (HCT 34.1-39%), or no anemia (HCT >39%), with no anemia serving as the reference group for all analyses. Independent associations between levels of anemia and postoperative outcomes were established using multivariable logistic regression. A sensitivity analysis was performed to assess interactions between preoperative anemia and blood transfusions.

Results

We identified 5,081 patients undergoing bypass, of which 741 (15%) had severe, 1,317 (26%) moderate, 1,516 (30%) mild, and 1,507 (30%) no anemia. Anemic patients were older and more commonly suffered from tissue loss and comorbidities (e.g. hypertension, diabetes, and renal insufficiency) (all P<.001). After adjustment for baseline conditions, mortality was higher in those with severe (3.1%, OR: 2.8, [95% CI: 1.3-6.3]) and moderate anemia (3.0%, OR: 2.6 [1.2-5.5]) compared to those without anemia (0.7%). Severe anemia was independently associated with major amputation (6.9% vs. 3.3%, OR: 1.6 [1.01-2.6]) compared to no anemia. Anemia on admission was additionally associated with several other adverse outcomes, such as major adverse cardiovascular event (MACE) (severe: OR: 1.9 [1.1-3.0]; moderate: OR: 1.9 [1.3-2.9]; mild: OR: 1.6 [1.1-2.4]) and unplanned return to the operating room (severe: OR: 1.6 [1.2-2.1]; moderate: OR: 1.5 [1.2-1.8]; mild: OR: 1.3 [1.03-1.6]). Moreover, mortality associated with preoperative anemia was not different in patients receiving postoperative blood transfusions compared to those who did not, while MACE was significantly higher in patients with preoperative anemia and blood transfusions (interaction; P<.001)

Conclusions

Mortality and major adverse events in CLTI patients undergoing infrainguinal bypass are inversely associated with preoperative hematocrit levels, with the highest event rates in the most severely anemic patients. The correlation between anemia and MACE – but not mortality – was stronger in those patients receiving postoperative blood transfusions. Further research is needed to define an appropriate transfusion threshold and attention should be focused on how to best optimize anemic CLTI patients prior to intervention.

INTRODUCTION

In patients undergoing surgery, it is estimated that nearly one-third suffer from anemia, and this number tends to be even higher in elderly patients and the critically ill.[1-3] As a marker of illness, anemia is associated with several unfavorable clinical and surgical outcomes.[4] Patients with chronic limb-threatening ischemia (CLTI) undergoing intervention represent a particularly fragile population with depleted reserves due to disease severity and existing comorbidities, and those with low hematocrit levels on admission may be at even higher risk.

Several studies have demonstrated an association between preoperative anemia and increased postoperative complications in cardiac and non-cardiac surgical patients, including mortality, major adverse cardiovascular event (MACE), wound infection, and prolonged hospital stay.[5-13] In the last few years, however, the focus has shifted more towards the risks and benefits of blood transfusions in patients with peripheral arterial disease. Despite the widespread use of blood transfusions in patients with anemia, several studies have failed to demonstrate a benefit and, in fact, blood transfusions have shown to be harmful in many clinical settings.[14-18] Some suggest that it is not the practice of treatment of anemia with blood transfusions that is associated with adverse events, but rather the anemia itself that increases the patients' risk of postoperative complications.[4] Interestingly, few reports have evaluated the combined effect of preoperative anemia and blood transfusions, although this is a relevant clinical question for physicians.[19,20] Detailed risk stratification could provide clinicians valuable guidance in optimizing anemic patients at the time of surgery.

Therefore, the aim of this study was to assess whether lower levels of hematocrit preoperatively were associated with postoperative adverse events in CLTI patients undergoing bypass surgery and determine whether this risk could be modified by blood transfusions.

METHODS

Data source

A retrospective cohort study was performed using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) targeted vascular module. NSQIP is a clinical registry designed to improve the quality of surgical care by prospective collection of clinical data that provides reliable and risk-adjusted surgical outcomes up to 30 days. The targeted vascular module captures a subset of patients with additional disease-specific and detailed anatomic characteristics, as well as procedure-related outcomes chosen by vascular surgeons. Trained clinical reviewers at each hospital identify potential procedures by reviewing operative case logs, categorize procedures using Current Procedural Terminology codes, and gather data in a standardized fashion according to strict definitions from both the targeted and non-targeted NSQIP. The reliability of NSQIP data collection has been validated previously.[21-23] More detailed information on this registry is available at

www.facs.org/quality-programs/acs-nsqip. We obtained approval from the Beth Israel Deaconess Medical Center Institutional Review Board without the need for informed consent because this registry contains de-identified data only.

Patients

All patients undergoing infrainguinal bypass between 2011 and 2014 were identified from the targeted vascular module. Emergent cases (N=389; 7.0%) and those missing preoperative hematocrit levels (N=52; 0.9%) were excluded from this study. Preoperative hematocrit was defined by NSQIP as the last hematocrit measurement prior to the index operation and hematocrit levels were obtained within one month of surgery in 97.4%, with a median of 1 day (IQR: 0-5) before surgery. Anemia was considered when the hematocrit level was 39.0% of blood volume or less, as specified by the World Health Organization.[24] The cohort was subsequently categorized in accordance with their preoperative hematocrit (HCT) levels into four arbitrary groups: severe anemia (HCT ≤ 29%), moderate anemia (HCT 29.1 – 34%), mild anemia (HCT 34.1 – 39%), and no anemia (HCT > 39%).

Variables

Baseline characteristics included demographics, comorbidities, and preprocedural medication. Additional concurrent procedures were evaluated, including endovascular interventions and endarterectomy. Postoperative (30-day) outcome variables were collected for all patients. In accordance with SVS efficacy measures[25], major adverse limb event (MALE) was defined as major amputation (above or below knee) and/or major reintervention (new bypass graft, thrombectomy or thrombolysis, or major surgical revision, such as jump or interposition graft), and MACE was defined as death from any cause, myocardial infarction, and/or stroke. Postoperative blood transfusion was defined as at least one unit of packed red blood cells during surgery or within 72 hours postoperatively and included blood transfused to patients collected by a cell saver. Neither the indication nor the hematocrit levels at the time of transfusion were available in NSQIP. Preoperative blood transfusion was also captured and refers to a transfusion during the 72 hours prior to surgery. Renal deterioration was defined as an increase in serum creatinine concentration of 2 mg/dL or more and/or the need for dialysis. Pulmonary complications included any unplanned reintubation, new pneumonia, or need for prolonged ventilatory support (longer than 48 hours postoperatively). Discharge other than home was only considered for those patients who were originally admitted from home. Variable definitions were not modifiable, as they were determined by NSQIP prior to data collection and can be found in the user guide (www.facs.org/quality-programs/acs-nsqip/program-specifics/participant-use).

Statistical analysis

Categorical variables are presented as counts and percentages. Normally distributed continuous variables are presented as mean ± standard deviation and variables with nonparametric distributions as median and interquartile range. Unadjusted comparisons

between groups were performed using Pearson's χ^2 and Fisher's exact test for categorical variables, and Student's t-test and Mann Whitney U test for continuous variables, where appropriate. Anemia was treated as a categorical variable with no anemia the reference group, and all P-values are relative to this group. Multivariable logistic regression models were constructed for independent associations between anemia and 30-day adverse outcomes, adjusting for similar covariates and confounders. Purposeful selection was used to determine covariates and confounders, which includes variables identified on univariate analysis ($P < .10$) and clinically relevant variables as described previously.[26]

Previous literature suggests that preoperative anemia is one of the strongest predictors of blood transfusion.[14,15] As such, blood transfusions are likely to act as an intermediate variable between the independent variable (preoperative anemia) and the dependent variable (adverse outcome). Due to this relationship, postoperative blood transfusion was not included as a covariate in the initial multivariable models which allowed more comprehensive assessment of the direct effect of anemia on outcomes. Additionally, a sensitivity analysis was performed to specifically assess effect modification between anemia and postoperative transfusion by forcing an interaction term in the multivariable regression models. Interactions enable the effect of transfusion to vary across the levels of anemia, which provides more detailed predictions. Since blood loss was not captured in this registry, we attempted to account for this in a subgroup analysis excluding those with postoperative blood transfusions, as this selected cohort presumably excludes the patients with significant bleeding. All statistical analyses were performed using SPSS Statistics 23 (IBM Corp, Armonk, NY) with $P < .05$ considered to be statistically significant. Figures were constructed using Prism 6 (GraphPad, La Jolla, CA).

RESULTS

A total of 5,081 patients with CLTI were included, of which 741 (15%) had severe anemia, 1,317 (26%) had moderate anemia, 1,516 (30%) had mild anemia, and 1,507 (30%) had no anemia.

Baseline and procedure details

The baseline demographics and comorbidities are summarized in Table I. Patients with preoperative anemia were considerably older and less likely to be male, white, or have a history of smoking. Patients with lower hematocrit levels on admission had more comorbid conditions, such as hypertension, diabetes, chronic heart failure, and renal insufficiency. Preoperative transfusion was more common among those with preoperative low hematocrit levels (severe: 9.9%, moderate: 4.3%, mild: 0.9%, no anemia: 0.1%; all $P < .01$). In addition, anemic patients more often had tissue loss compared to those without anemia (severe: 77%, moderate: 69%, mild: 58%, no anemia: 40%; all $P < .001$).

Table I. Baseline characteristics for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery

	No anemia		**Severe anemia**			**Moderate anemia**			**Mild anemia**		
	(> 39%)		**(≤ 29%)**		**P-value vs. no anemia**	**(29.1 – 34%)**		**P-value vs. no anemia**	**(34.1 – 39%)**		**P-value vs. no anemia**
	(N=1507)		*(N=741)*			*(N=1317)*			*(N=1516)*		
	N	*%*	*N*	*%*		*N*	*%*		*N*	*%*	
Male gender	1127	(75)	393	(53)	**<.001**	703	(53)	**<.001**	932	(62)	**<.001**
Age - years (mean ± SD)	65.7 (11)		69.1 (11)		**<.001**	70.0 (12)		**<.001**	69.1 (12)		**<.001**
Age categories					**<.001**			**<.001**			**<.001**
< 60	451	(30)	149	(20)		249	(19)		331	(22)	
60 to 69	528	(35)	219	(30)		378	(29)		441	(29)	
70 to 79	341	(23)	227	(31)		376	(29)		420	(28)	
80 to 89	165	(11)	133	(18)		265	(20)		286	(19)	
> 89	22	(1.5)	13	(1.8)		49	(3.7)		38	(2.5)	
Smoking	828	(55)	234	(32)	**<.001**	404	(31)	**<.001**	554	(37)	**<.001**
BMI > 30	456	(31)	203	(28)	.20	363	(28)	.14	442	(30)	.54
Race					**<.001**			**<.001**			**<.001**
White	1087	(72)	376	(51)		792	(60)		977	(64)	
Black	222	(15)	233	(31)		313	(24)		305	(20)	
Hispanic	60	(4.0)	47	(6.3)		84	(6.4)		74	(4.9)	
Other	14	(0.9)	19	(2.6)		23	(1.7)		19	(1.3)	
Unknown/not reported	124	(8.2)	66	(8.9)		105	(8.0)		141	(9.3)	
Symptom status					**<.001**			**<.001**			**<.001**
Rest pain	907	(60)	171	(23)		409	(31)		630	(42)	
Tissue loss	600	(40)	511	(77)		908	(69)		886	(58)	
Prior ipsilateral revascularization	606	(40)	299	(40)	.95	510	(39)	.42	594	(39)	.56
Not admitted from home	110	(7.3)	144	(19)	**<.001**	229	(17)	**<.001**	164	(11)	**.001**
Dependent functional status	60	(4.0)	83	(11)	**<.001**	175	(13)	**<.001**	146	(9.7)	**<.001**
Hypertension	1176	(78)	658	(89)	**<.001**	1147	(87)	**<.001**	1287	(85)	**<.001**
Diabetes	555	(37)	455	(61)	**<.001**	786	(60)	**<.001**	779	(51)	**<.001**
CHF	26	(1.7)	49	(6.6)	**<.001**	68	(5.2)	**<.001**	32	(2.1)	.44
Renal Insufficiency	184	(12)	339	(46)	**<.001**	436	(33)	**<.001**	336	(22)	**<.001**
Dialysis	25	(1.7)	128	(17)	**<.001**	152	(12)	**<.001**	97	(6.4)	**<.001**
COPD	206	(14)	90	(12)	.32	156	(12)	.15	202	(13)	.78

Table I. *Continued*

	No anemia **(> 39%)** *(N=1507)*		**Severe anemia** **(≤ 29%)** *(N=741)*		**P-value vs. no anemia**	**Moderate anemia** **(29.1 – 34%)** *(N=1317)*		**P-value vs. no anemia**	**Mild anemia** **(34.1 – 39%)** *(N=1516)*		**P-value vs. no anemia**
	N	*%*	*N*	*%*		*N*	*%*		*N*	*%*	
Preprocedural medication											
Antiplatelet	1200	(80)	587	(80)	.94	1056	(81)	.36	1218	(81)	.55
Statin	977	(65)	523	(71)	**<.01**	963	(74)	**<.001**	1057	(70)	**<.01**
Beta-blocker	830	(55)	537	(73)	**<.001**	901	(69)	**<.001**	953	(63)	**<.001**
Transfusion <72h before surgery	1	(0.1)	73	(9.9)	**<.001**	56	(4.3)	**<.001**	13	(0.9)	**<.01**
Preoperative open wound/infection	499	(33)	476	(64)	**<.001**	808	(61)	**<.001**	730	(48)	**<.001**
Hematocrit - Vol% (median ± IQR)	42 (41-45)		27 (26-28)		**<.001**	32 (31-33)		**<.001**	37 (35-38)		**<.001**

SD: standard deviation, *BMI*: body mass index, *CHF*: congestive heart failure, *COPD*: chronic obstructive pulmonary disease, *h*: hours, *IQR*: interquartile range

Regarding operative characteristics, patients with preoperative anemia more often underwent infrapopliteal procedures, whereas femoropopliteal bypass was more common in non-anemic patients (Table II). Additionally, anemic patients were less likely to undergo elective procedures, more frequently had an ASA class ≥ 4 (severe: 35%, moderate: 33%, mild: 25%, no anemia: 17%; all P < .001), and had longer procedure times (severe: 247 minutes, [IQR: 191-327], moderate: 233 [179-315], no anemia: 221 [165-296]; all P < .001). No differences were observed in the frequency of concurrent procedures between patients with and without anemia.

Postoperative outcomes

Postoperative outcomes are detailed in Table III A. Thirty-day mortality was substantially higher among patients with the lowest hematocrit levels and declined with increasing hematocrit levels (severe: 3.1%, moderate: 3.0%, mild: 1.8%, no anemia: 0.7%; all P < .01, Figure 1). In addition, patients with severe anemia had higher 30-day amputation rates than those without anemia (6.9% vs. 3.3%, P < .001). MACE was significantly more common in anemic patients compared to those without anemia (severe: 6.9%, moderate: 6.8%, mild: 5.1%, no anemia: 2.6%; all P < .001). Preoperative anemia was also associated with higher rates of several other postoperative adverse events, including septic shock, renal deterioration, pulmonary complications, unplanned return to the operating room, and unplanned readmissions. Furthermore, postoperative blood transfusions were more frequent in those patients with preoperative low hematocrit levels (severe: 67%, moderate:

Table II. Procedure details for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery

	No anemia (> 39%) *(N=1507)*		**Severe anemia (≤ 29%)** *(N=741)*		**P-value vs. no anemia**	**Moderate anemia (29.1 – 34%)** *(N=1317)*		**P-value vs. no anemia**	**Mild anemia (34.1 – 39%)** *(N=1516)*		**P-value vs. no anemia**
	N	*%*	*N*	*%*		*N*	*%*		*N*	*%*	
Type of bypass procedure					**<.001**			**<.001**			**<.01**
Femoropopliteal bypass	882	(59)	351	(47)		683	(52)		811	(54)	
Femoral-tibial/pedal bypass	499	(33)	268	(36)		473	(36)		532	(35)	
Popliteal-tibial/pedal bypass	126	(8.4)	122	(17)		161	(12)		173	(11)	
Graft type					.53			.25			**.03**
Saphenous vein	928	(62)	446	(60)		783	(60)		876	(58)	
Prosthetic/spliced vein/ composite	579	(38)	295	(40)		534	(40)		640	(42)	
Concurrent procedures											
Endovascular interventions	78	(5.2)	30	(4.0)	.24	54	(4.1)	.18	65	(4.3)	.25
Endarterectomy	179	(13)	85	(12)	.28	205	(16)	.06	203	(13)	.80
Elective procedure	1139	(76)	291	(39)	**<.001**	639	(49)	**<.001**	944	(62)	**<.001**
ASA class ≥ 4	253	(17)	262	(35)	**<.001**	429	(33)	**<.001**	383	(25)	**<.001**
Overall procedure time – min (median ± IQR)	221 (165-296)		247 (191-327)		**<.001**	233 (179-315)		**<.001**	227 (171-307)		.08

ASA: American Society of Anesthesiologists, *min*: minutes, *IQR*: interquartile range

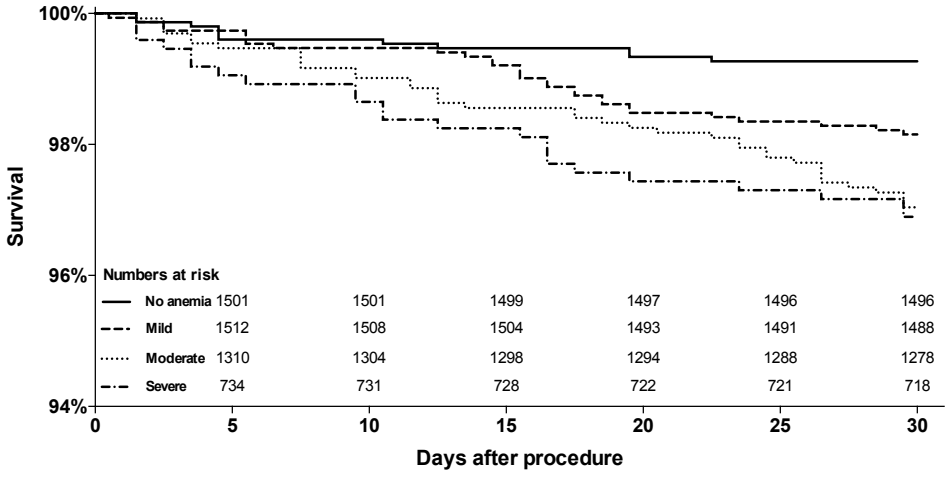


Figure 1. Unadjusted survival probabilities for patients with chronic limb-threatening ischemia (CLTI) based on anemia categories

43%, mild: 25%, no anemia: 11%; all P <. 001). No differences were found in MALE or surgical site infections. Finally, hospital length of stay was significantly longer among patients with preoperative anemia compared to those with no anemia (severe: 12 days, [IQR: 8-18], moderate: 9 [5-15], mild: 7 [4-11], no anemia: 5 [3-7]; all P < .001).

In an attempt to account for blood loss and directly assess the effect of anemia, we performed an additional subgroup analysis comparing 30-day outcomes by the level of anemia in those who did not receive postoperative blood transfusions (N=3,482). As detailed in Table III B, preoperative anemia was again associated with higher rates of mortality, major amputation, MACE, septic shock, and unplanned reoperations on unadjusted analyses.

Table III A. Postoperative outcomes for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery

	No anemia (> 39%) *(N=1507)*		**Severe anemia (≤ 29%)** *(N=741)*		**P-value vs. no anemia**	**Moderate anemia (29.1 – 34%)** *(N=1317)*		**P-value vs. no anemia**	**Mild anemia (34.1 – 39%)** *(N=1516)*		**P-value vs. no anemia**
	N	*%*	*N*	*%*		*N*	*%*		*N*	*%*	
Mortality	11	(0.7)	23	(3.1)	**<.001**	39	(3.0)	**<.001**	28	(1.8)	**<.01**
MALE	131	(8.7)	77	(10)	.19	109	(8.3)	.69	113	(7.5)	.21
Major amputation	50	(3.3)	51	(6.9)	**<.001**	58	(4.4)	.13	44	(2.9)	.51
Major reintervention	94	(6.2)	39	(5.3)	.36	65	(4.9)	.13	81	(5.3)	.29
MACE	39	(2.6)	51	(6.9)	**<.001**	89	(6.8)	**<.001**	78	(5.1)	**<.001**
Surgical site infection	133	(8.8)	57	(7.7)	.36	104	(7.9)	.38	137	(9.0)	.84
Superficial	93	(6.2)	45	(6.1)	.93	72	(5.5)	.43	94	(6.2)	.97
Deep	38	(2.5)	9	(1.2)	**.04**	30	(2.3)	.67	39	(2.6)	.93
Organ space	3	(0.2)	4	(0.5)	.23	4	(0.3)	.58	5	(0.3)	.73
Wound dehiscence	37	(2.5)	5	(0.7)	**<.01**	24	(1.8)	.25	33	(2.2)	.61
Transfusion (≥1 RBPC)	164	(11)	498	(67)	**<.001**	561	(43)	**<.001**	376	(25)	**<.001**
Septic shock	7	(0.5)	16	(2.2)	**<.001**	15	(1.1)	**.04**	7	(0.5)	.99
Renal deterioration	12	(0.8)	15	(2.0)	**.01**	23	(1.7)	**.02**	17	(1.1)	.36
Requiring dialysis	5	(0.3)	7	(0.9)	.06	11	(0.8)	.08	8	(0.5)	.41
Pulmonary complication	26	(1.7)	33	(4.5)	**<.001**	56	(4.3)	**<.001**	51	(3.4)	**<.01**
Unplanned return to OR	215	(14)	172	(23)	**<.001**	267	(20)	**<.001**	268	(18)	**.01**
Untreated loss of patency	34	(2.3)	23	(3.1)	.23	45	(3.4)	.06	36	(2.4)	.83
Discharge other than home	288	(20)	345	(53)	**<.001**	449	(38)	**<.001**	438	(31)	**<.001**
Length of stay - d (median ± IQR)	5 (3-7)		12 (8-18)		**<.001**	9 (5-15)		**<.001**	7 (4-11)		**<.001**
Unplanned readmissions	222	(15)	156	(21)	**<.001**	253	(19)	**<.01**	291	(19)	**.001**

MALE: major adverse limb event, *MACE*: major adverse cardiovascular event, *RBPC*: red blood packed cells, *OR*: operating room, *d*: days, *IQR*: interquartile range

Table III B. Postoperative outcomes for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery, restricted to those without any postoperative blood transfusion (N=3482)

	No postoperative transfusion										
	No anemia		**Severe anemia**			**Moderate anemia**			**Mild anemia**		
	(> 39%)		**(≤ 29%)**		**P-value vs. no anemia**	**(29.1 – 34%)**		**P-value vs. no anemia**	**(34.1 – 39%)**		**P-value vs. no anemia**
	(N=1343)		*(N=243)*			*(N=756)*			*(N=1140)*		
	N	*%*	*N*	*%*		*N*	*%*		*N*	*%*	
Mortality	5	(0.4)	9	(3.7)	**<.001**	15	(2.0)	**<.001**	17	(1.5)	**<.01**
MALE	101	(7.5)	21	(8.6)	.55	58	(7.7)	.90	69	(6.1)	.15
Major amputation	38	(2.8)	14	(5.8)	**.02**	34	(4.5)	**.04**	28	(2.5)	.56
Major reintervention	74	(5.5)	12	(4.9)	.72	32	(4.2)	.20	48	(4.2)	.14
MACE	22	(1.6)	11	(4.5)	**<.01**	34	(4.5)	**<.001**	44	(3.9)	**.001**
Surgical site infection	115	(8.6)	15	(6.2)	.21	44	(5.8)	**.02**	97	(8.5)	.96
Superficial	83	(6.2)	10	(4.1)	.21	29	(3.8)	**.02**	68	(6.0)	.82
Deep	30	(2.2)	2	(0.8)	.21	13	(1.7)	.43	27	(2.4)	.82
Organ space	3	(0.2)	3	(1.2)	.05	2	(0.3)	>.99	3	(0.3)	>.99
Wound dehiscence	32	(2.4)	1	(0.4)	**.049**	15	(2.0)	.55	20	(1.8)	.28
Septic shock	5	(0.4)	6	(2.5)	**<.001**	3	(0.4)	>.99	4	(0.4)	>.99
Renal deterioration	10	(0.7)	5	(2.1)	.05	9	(1.2)	.30	11	(1.0)	.55
Requiring dialysis	4	(0.3)	5	(2.1)	**<.01**	5	(0.7)	.22	4	(0.4)	>.99
Pulmonary complication	16	(1.2)	8	(3.3)	**.01**	21	(2.8)	**<.01**	26	(2.3)	**.04**
Unplanned return to OR	176	(13)	48	(20)	**<.01**	143	(19)	**<.001**	182	(16)	**.04**
Untreated loss of patency	27	(2.0)	5	(2.1)	>.99	26	(3.4)	**.045**	24	(2.1)	.86
Discharge other than home	228	(17)	90	(41)	**<.001**	223	(33)	**<.001**	290	(27)	**<.001**
Length of stay - d (median ± IQR)	4 (3-7)		11 (7-18)		**<.001**	8 (5-14)		**<.001**	6 (4-11)		**<.001**
Unplanned readmissions	189	(14)	42	(17)	.19	132	(18)	**.04**	215	(19)	**.001**

MALE: major adverse limb event, *MACE*: major adverse cardiovascular event, *OR*: operating room, *d*: days, *IQR*: interquartile range

Multivariable analysis

After adjustment for potential confounders, both severe and moderate anemia were associated with 30-day mortality compared to those with normal hematocrit levels (OR: 2.8, [95% CI: 1.3-6.3] and OR: 2.6 [1.2-5.5], respectively) (Table IV A). Among patients with CLTI, severe anemia also proved to be associated with major amputation in the first 30-days postoperatively (OR: 1.6 [1.01-2.6]). In addition, lower levels of hematocrit preoperatively were associated with MACE (severe: OR: 1.9 [1.1-3.0]; moderate: OR: 1.9 [1.3-2.9]; mild: OR: 1.6 [1.1-2.4]), renal deterioration (severe: OR: 2.5 [1.05-5.9]; moderate: OR: 2.3 [1.1-4.9]), unplanned return to the operating room (severe: OR: 1.6 [1.2-2.1]; moderate: OR: 1.5 [1.2-1.8]; mild: OR: 1.3 [1.03-1.6]), and unplanned readmission (severe: OR: 1.4 [1.1-1.8]; moderate: OR: 1.3 [1.02-1.6]; mild: OR: 1.3 [1.1-1.6]).

Table IV A. Multivariable analysis for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery with the reference group no anemia (HCT > 39%)

	Severe anemia (≤ 29%) *(N=741)*		**Moderate anemia (29.1 – 34%)** *(N=1317)*		**Mild anemia (34.1 – 39%)** *(N=1516)*	
	OR	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Mortality	**2.8**	**(1.3-6.3)**	**2.6**	**(1.2-5.5)**	1.9	(0.9-4.0)
Major amputation	**1.6**	**(1.01-2.6)**	1.2	(0.8-1.8)	0.8	(0.5-1.2)
MACE	**1.9**	**(1.1-3.0)**	**1.9**	**(1.3-2.9)**	**1.6**	**(1.1-2.4)**
Renal deterioration	**2.5**	**(1.04-5.9)**	**2.3**	**(1.1-4.9)**	1.5	(0.7-3.3)
Unplanned return to OR	**1.6**	**(1.2-2.1)**	**1.5**	**(1.2-1.8)**	**1.3**	**(1.03-1.6)**
Unplanned readmissions	**1.4**	**(1.1-1.8)**	**1.3**	**(1.02-1.6)**	**1.3**	**(1.1-1.6)**

CI: Confidence interval, *OR*: odds ratio, *HCT*: hematocrit, *MACE*: major adverse cardiovascular event.
Adjusted for age, sex, race, symptom status, smoking, obesity, preoperative transfusion, hypertension, diabetes, congestive heart failure, renal insufficiency, preoperative dialysis, and type of procedure.

Among patients who did not receive postoperative blood transfusions, severe anemia was still significantly associated with 30-day mortality (OR: 5.8 [1.6-21.5]), while in those with moderate and mild anemia, a nonsignificant trend toward higher mortality was observed (OR: 3.1 [0.95-10.1] and OR: 2.9 [0.9-8.8], respectively) (Table IV B). Similar associations and odds ratios to the larger cohort were observed for several other adverse outcomes, although some became nonsignificant in the smaller sample.

Table IV B. Multivariable analysis for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery with the reference group no anemia (HCT > 39%), restricted to those without any postoperative blood transfusion (N=3482)

	No postoperative transfusion					
	Severe anemia (≤ 29%) *(N=243)*		**Moderate anemia (29.1 – 34%)** *(N=756)*		**Mild anemia (34.1 – 39%)** *(N=1140)*	
	OR	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Mortality	**5.8**	**(1.6-21.5)**	3.1	(0.95-10.1)	2.9	(0.9-8.8)
Major amputation	1.5	(0.7-3.1)	1.3	(0.8-2.2)	0.7	(0.4-1.2)
MACE	1.6	(0.7-3.7)	1.8	(0.98-3.3)	**1.8**	**(1.03-3.1)**
Renal deterioration	*		*		*	
Unplanned return to OR	**1.6**	**(1.1-2.3)**	**1.5**	**(1.2-1.9)**	1.2	(0.98-1.6)
Unplanned readmissions	1.1	(0.7-1.6)	1.1	(0.9-1.5)	**1.4**	**(1.1-1.7)**

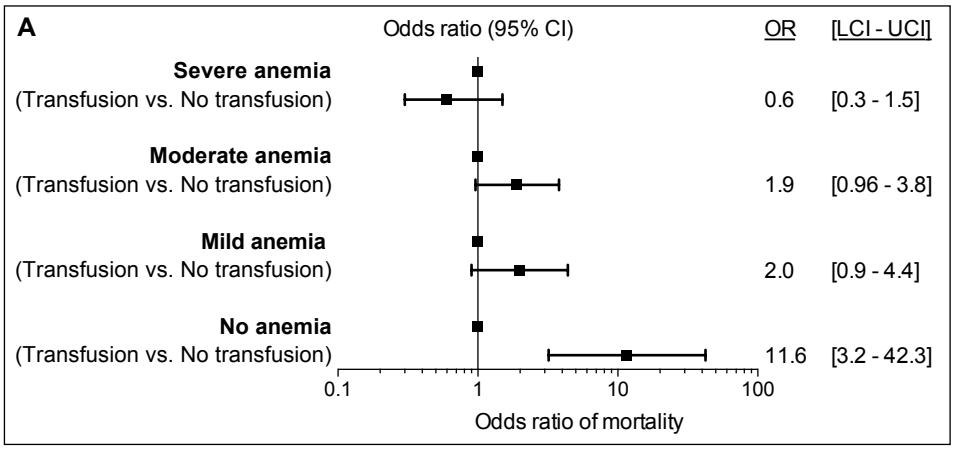
CI: Confidence interval, *OR*: odds ratio, *HCT*: hematocrit, *MACE*: major adverse cardiovascular event.
Adjusted for age, sex, race, symptom status, smoking, obesity, preoperative transfusion, hypertension, diabetes, congestive heart failure, renal insufficiency, preoperative dialysis, and type of procedure.
* too few events

Effect modification

A sensitivity analysis was performed by incorporating effect modification between anemia and postoperative blood transfusion in the multivariable models. Adjusted odds ratios were compared within anemia categories, with no transfusion as the reference group (Figure 2). Although the interaction term was significant (P = .001), 30-day mortality was not different in anemic patients comparing those who received postoperative blood transfusions to those without any blood transfusions and comparable hematocrit levels. However, in patients with the lowest hematocrit levels, transfusion demonstrated a trend toward reduced mortality (within severe anemia, transfusion vs. no transfusion: OR: 0.6 [0.3-1.5]). Mortality was notably more pronounced in non-anemic patients receiving postoperative blood transfusions (OR: 11.6 [3.2-42.3]).

In the adjusted analysis, postoperative blood transfusions were associated with MACE in patients with preoperative anemia when compared to those that did not receive any blood transfusions (interaction P < .001). Similarly, MACE was higher in patients with normal hematocrit levels preoperatively and blood transfusions (OR: 7.4 [3.8-14.5]).

Of note, transfusion prior to surgery showed a protective trend in all models (OR < 1.0), e.g. mortality (OR: 0.5 [0.2-1.1]) and MACE (OR: 0.8 [0.4-1.4]). Unadjusted adverse event rates of CLTI patients with different degrees of anemia stratified by postoperative blood transfusion are detailed in Supplementary table I.



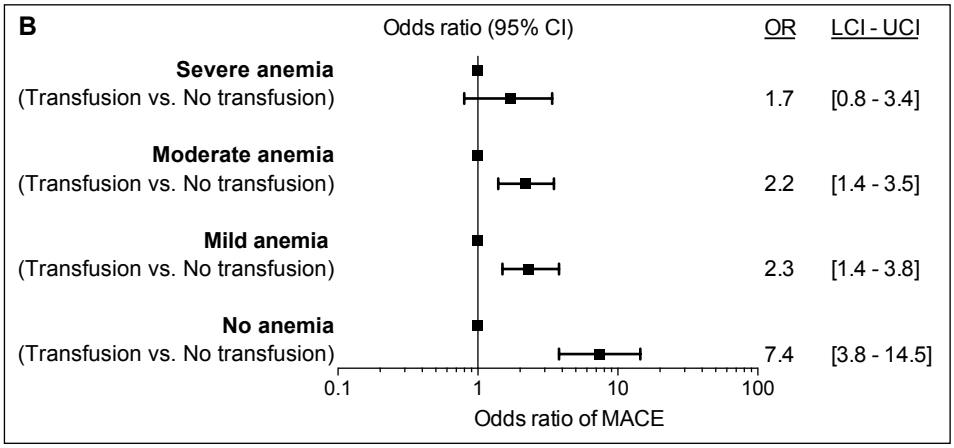


Figure 2. Multivariable analysis for patients with chronic limb-threatening ischemia (CLTI) incorporated with effect modification between anemia and postoperative blood transfusions for 30-day mortality (**A**) and MACE (**B**)

CI: Confidence interval, *OR*: odds ratio, *LCI*: lower confidence interval, *UCI*: upper confidence interval, *MACE*: major adverse cardiovascular event. Adjusted for age, sex, race, symptom status, smoking, obesity, preoperative transfusion, hypertension, diabetes, congestive heart failure, renal insufficiency, preoperative dialysis, and type of procedure

DISCUSSION

This study demonstrates that mortality increases in CLTI patients undergoing infrainguinal bypass as preoperative hematocrit level decreases. Mortality within 30 days was 2.8-fold higher in those with severe anemia and these patients were 1.6 times more likely to undergo a major amputation compared to those without anemia. In addition, even the milder degrees of preoperative anemia were associated with mortality and MACE. Some of these worse outcomes were mitigated by higher transfusion rates in the more anemic patients; however, by analyzing non-transfused patients only in an attempt to account for blood loss, we still demonstrated a higher mortality risk (5.8-fold) associated with severe anemia. Interestingly, MACE was higher in anemic patients that received postoperative blood transfusions when compared to those without any blood transfusions, likely reflecting the impact of bleeding or the detrimental effect of perioperative blood transfusions. Mortality in those with preoperative anemia was not affected by blood transfusion.

As in critical care and cardiac surgery, patients with preoperative anemia undergoing vascular interventions have a higher risk of early and late mortality and morbidity.[6,11,27-30] Gupta et al. demonstrated that preoperative anemia (HCT < 39%) was associated with a higher rate of 30-day mortality (2.4% vs. 1.2%) and cardiac events (2.3% vs. 1.2%) compared to non-anemic elderly patients undergoing elective vascular procedures in NSQIP.[28] A retrospective analysis of patients between 1990-2006 from a single institution also reported that the degree of anemia was inversely associated with not only 30-day but also 5-year MACE in 1,211 vascular patients.[6] Although we found similar short-term associations in our adjusted analysis, the above-mentioned studies lack granularity by including various vascular procedures (e.g. carotid surgery, abdominal aneurysm repair, and peripheral bypass) without adjusting for procedural information or disease severity. In contrast, Veluscu et al. studied an institutional cohort of 403 patients with CLTI undergoing revascularization, and demonstrated that preoperative anemia (hemoglobin concentration of < 10 g/dL) was associated with 30-day mortality (17.7% vs. 5.1%, OR: 3.9 [1.8-8.4]) compared to those without anemia.[30] Although they had an older cohort (mean age 73 vs. 68 years in our study), the mortality rates within 30 days were significantly higher than the present study (anemia 1.8-3.1% vs. no anemia: 0.7%), which is likely related to sample variability and simply a result of single-institution data and a smaller sample size. Since NSQIP is a large and nationally representative registry, we believe our data provide more generalizable risk estimations.

Our data additionally suggest that postoperative blood transfusions were associated with an increased risk of MACE in anemic CLTI patients, whereas transfusions did not affect 30-day mortality, although there was a trend toward lower mortality in patients with severe anemia. In 2010, Wu et al. studied a large cohort of 239,286 elderly patients undergoing major non-cardiac surgery from the Department of Veterans Affairs NSQIP registry and, using propensity score matching, demonstrated that 30-day mortality was higher in transfused patients with hematocrit levels of ≥ 30% (OR: 1.4 [1.2-1.5]) but lower

in those with hematocrit levels of < 24% (OR: 0.6 [0.4-0.9]) as opposed to patients with comparable hematocrit levels without transfusion.[20] In a subgroup analysis, the authors accounted for estimated intraoperative blood loss derived from an alternative anesthesiology formula. The hematocrit threshold for mortality benefit or risk did not change in patients with a blood loss of less than 500 mL. Yet, in patients with substantial intraoperative blood loss (500 – 999 mL), mortality was lower in those with hematocrit values between 30% and 53.9% receiving blood transfusions as opposed to those that did not. It is important to note that estimated blood loss was not captured in the current study, although we attempted to account for this by excluding patients with postoperative blood transfusions, which presumably represents a cohort without significant bleeding. Blood transfusions in the less severely anemic patients are more likely due to blood loss rather than the sequela of anemia, which is more likely the indication to transfuse those with severe anemia. Thus, the results of our subgroup analysis suggest that, first and foremost, the most severely anemic patients tend to do much worse with significantly lower survival. In addition, transfusions, or the perceived need for transfusions due to bleeding, were associated with a higher risk of adverse events. However, more detailed data with hematocrit at the time of transfusion and the indication of ongoing blood loss would be necessary to accurately determine this exact correlation.

In the cardiac and vascular surgery literature, similar unfavorable outcomes associated with transfusions are described in patients undergoing a variety of procedures.[14-16,31] The large number of CLTI patients with anemia highlights the need for clinical practice guidelines, yet recommendations from vascular societies currently do not exist. Our observations reflect the lack of a uniform standard to guide the appropriateness of blood product usage, as hematocrit values that triggered transfusions varied greatly. Other medical societies favor restrictive transfusion practices increasingly over liberal approaches.[32,33] Practice guidelines from the American Association of Blood Banks (AABB) recommend a restrictive transfusion strategy in postoperative surgical patients as well as in those with preexisting cardiovascular disease with a hemoglobin concentration threshold of 8 g/dL.[32] Therefore, taking the extensive cardiovascular burden of CLTI patients into consideration, together with the findings of the present study, a similarly restrictive approach could be beneficial in the CLTI population. While we could not incorporate blood loss into the equation, further studies are needed to define an optimal threshold for blood transfusions and confirm our findings in other registries specific to patients with CLTI.

Since perioperative correction of anemia with blood transfusions is not free of risk, investigation of anemia prior to the surgical incision is appropriate whenever possible. Preoperative screening 4 weeks before an elective intervention may detect anemia in a timely manner allowing adequate time to respond. While delaying surgery may likely not be the best treatment in CLTI patients, particularly those with tissue loss, a compelling argument may be that preoperative correction of anemia could prevent postoperative blood transfusions, which likely results in a mortality benefit. This is supported by the fact that we observed a protective trend of blood transfusions prior to surgery in our multivariable

models. We believe that this finding emphasizes the need to investigate the appropriate role of preoperative blood transfusions. However, alternative options to treat anemia should be explored where appropriate, such as correction of nutritional deficiencies (iron deficiency reported in 32% of CLTI patients)[34] or erythropoiesis-stimulating agents.

Several study limitations should be considered in the interpretation of these data. While this study established an association between preoperative anemia and various adverse events, in a retrospective cohort study association does not necessarily imply causation. In addition, it remains a challenge to conclude whether anemia is an independent risk factor for worse outcome, or a marker of critical illness (e.g. renal failure) and disease severity, although we attempted to account for this in the multivariable analysis. With the NSQIP registry we are limited by variable definitions and, unfortunately, we could not account for unmeasured confounders, such as the units of red blood cells used. Additionally, hematocrit levels were obtained prior to surgery and postoperative values, or at the time of transfusion, were not documented, subsequently precluding their consideration in the multivariable analysis. Finally, long-term outcomes could not be assessed with this registry. Nonetheless, despite these limitations, the targeted module provides a large cohort containing granular detail specifically related to patients with CLTI.

CONCLUSIONS

This study highlights the associated risk of preoperative anemia in patients with CLTI as well as the hazards of postoperative blood transfusions as they are intimately related. Adverse events were inversely associated with preoperative hematocrit levels and, although we could not adjust for estimated blood loss, these data suggest that postoperative blood transfusions did not reduce – but rather increase – postoperative risks in the less severely anemic patients. Additional studies are warranted to evaluate optimization of hematocrit prior to surgery and, since we cannot yet justify widespread utilization of allogeneic blood transfusions, efforts should be directed towards assessment of an appropriate threshold for perioperative transfusions in CLTI patients undergoing revascularization.

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Supplementary table I. Postoperative outcomes stratified by anemia category and postoperative transfusion for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass surgery

	No anemia (> 39%) *(N=1507)*			**Severe anemia (≤ 29%)** *(N=741)*			**Moderate anemia (29.1 – 34%)** *(N=1317)*			**Mild anemia (34.1 – 39%)** *(N=1516)*		
	Transfusion *N=164* (11%)	**No Transfusion** *N=1343* (89%)		**Transfusion** *N=498* (67%)	**No Transfusion** *N=243* (33%)		**Transfusion** *N=561* (43%)	**No Transfusion** *N=756* (57%)		**Transfusion** *N=376* (25%)	**No Transfusion** *N=1140* (75%)	
Major adverse events			**P-value**			**P-value**			**P-value**			**P-value**
Mortality	6 (3.7)	5 (0.4)	**<.001**	14 (2.8)	9 (3.7)	.511	24 (4.3)	15 (2.0)	**.015**	11 (2.9)	17 (1.5)	.073
MALE	30 (18.3)	101 (7.5)	**<.001**	56 (11.2)	21 (8.6)	.276	51 (9.1)	58 (7.7)	.355	44 (11.7)	69 (6.1)	**<.001**
Major amputation	12 (7.3)	38 (2.8)	**.002**	37 (7.4)	14 (5.8)	.400	24 (4.3)	34 (4.5)	.848	16 (4.3)	28 (2.5)	.072
Major reintervention	20 (12.2)	74 (5.5)	**.001**	27 (5.4)	12 (4.9)	.782	33 (5.9)	32 (4.2)	.172	33 (8.8)	48 (4.2)	**.001**
MACE	17 (10.4)	22 (1.6)	**<.001**	40 (8.0)	11 (4.5)	.077	55 (9.8)	34 (4.5)	**<.001**	34 (9.0)	44 (3.9)	**<.001**
Surgical site infection	18 (11.0)	115 (8.6)	.304	42 (8.4)	15 (6.2)	.278	60 (10.7)	44 (5.8)	**.001**	40 (10.6)	97 (8.5)	.212
Wound dehiscence	5 (3.0)	32 (2.4)	.603	4 (0.8)	1 (0.4)	>.99	9 (1.6)	15 (2.0)	.610	13 (3.5)	20 (1.8)	.050
Septic shock	2 (1.2)	5 (0.4)	.172	10 (2.0)	6 (2.5)	.685	12 (2.1)	3 (0.4)	**.006**	3 (0.8)	4 (0.4)	.374
Renal deterioration	2 (1.2)	10 (0.7)	.382	10 (2.0)	5 (2.1)	.964	14 (2.5)	9 (1.2)	.074	6 (1.6)	11 (1.0)	.314
Requiring dialysis	1 (0.6)	4 (0.3)	.438	2 (0.4)	5 (2.1)	**.041**	6 (1.1)	5 (0.7)	.421	4 (1.1)	4 (0.4)	.111
Pulmonary complication	10 (6.1)	16 (1.2)	**<.001**	25 (5.0)	8 (3.3)	.284	35 (6.2)	21 (2.8)	**.002**	25 (6.6)	26 (2.3)	**<.001**
Unplanned reoperations	39 (23.8)	176 (13.1)	**<.001**	124 (24.9)	48 (19.8)	.119	124 (22.1)	143 (18.9)	.155	86 (22.9)	182 (16.0)	**.002**
Discharge details												
Untreated loss of patency	7 (4.3)	27 (2.0)	.066	18 (3.6)	5 (2.1)	.251	19 (3.4)	26 (3.4)	.959	12 (3.2)	24 (2.1)	.230
Discharge other than home	60 (37.5)	228 (17.4)	**<.001**	217 (50.1)	90 (41.1)	**.029**	226 (45.1)	223 (32.7)	**<.001**	148 (42.2)	290 (26.8)	**<.001**
Length of stay - d (median ± IQR)	7 (4-10)	4 (3-7)	**<.001**	12 (8-18)	11 (7-18)	.225	10 (6-15)	8 (5-14)	**<.001**	8 (5-13)	6 (4-11)	**<.001**
Unplanned readmissions	33 (20.1)	189 (14.1)	**.039**	114 (22.9)	42 (17.3)	.079	121 (21.6)	132 (17.5)	.061	76 (20.2)	215 (18.9)	.563

MALE: major adverse limb event, *MACE:* major adverse cardiovascular event, *d:* days, *IQR:* interquartile range

CHAPTER 6

LONG-TERM OUTCOMES AFTER INFRAINGUINAL BYPASS IN ANEMIC PATIENTS WITH CHRONIC LIMB-THREATENING ISCHEMIA

Submitted for publication

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ABSTRACT

Objective

Preoperative anemia and blood transfusions are common risk factors for adverse events in patients with chronic limb-threatening ischemia (CLTI); however, their complex interaction is largely unknown. The purpose of this study was to assess associations between varying severities of anemia and short- as well as long-term outcomes, and determine whether these outcomes were influenced by blood transfusions.

Methods

The Vascular Study Group of New England registry was queried from 2008 to 2015 for all non-emergent infrainguinal bypass procedures for CLTI. Patients were compared based on their preoperative hemoglobin concentration: moderate/severe (≤9 g/dL), mild (male: 9.1-12.9 g/dL, female: 9.1-11.9 g/dL), or no anemia (male: ≥13 g/dL, female: ≥12 g/dL). Outcomes included in-hospital cardiac complications and 1-year mortality and limb events. Multivariable analysis was used to determine independent associations between levels of anemia and adverse events. A second analysis was performed to assess effect modification between anemia and blood transfusions.

Results

Of the 2,822 patients, 219 (7.8%) presented with moderate/severe, 1,376 (49%) with mild, and 1,227 (43%) without preoperative anemia. Anemic patients were older and had more tissue loss and coexisting conditions (e.g. coronary artery disease, diabetes, and end-stage renal disease) (all P<.001). After adjusting for baseline characteristics, preoperative anemia was not associated with in-hospital cardiac complications (moderate/severe: OR 1.27 [0.77-2.10], mild: OR 1.29 [0.95-1.74]). Conversely, lower hemoglobin levels on admission were associated with higher one-year mortality (moderate/severe: 21%, HR 2.17 [1.48-3.20], mild: 17%, HR 1.87 [1.43-2.45]) compared to those without anemia (6.5%). Similar estimates were found for one-year major amputation or death (moderate/severe: HR 2.00 [1.48-2.71], mild: HR 1.48 [1.20 – 1.83], respectively), while only moderate/severe anemia was a risk factor for major amputation (HR 2.25 [1.42-3.57]). Blood transfusions in non-anemic patients were associated with higher one-year mortality, primarily found in patients above age 75 and without excessive operative blood loss (<400 mL), whereas transfusion was not shown to affect mortality in anemic patients, compared to those not receiving blood transfusion. Among both anemic and non-anemic patients, those receiving blood transfusions had higher in-hospital cardiac complications.

Conclusions

Preoperative anemia in patients with CLTI was associated with late mortality and limb events, whereas in-hospital cardiac complications were not affected by anemia but rather by transfusion. As such, physicians should carefully deliberate blood product usage and avoid transfusions in milder degrees of anemia whenever possible, as well as create awareness of alternatives to transfusion even for anemia of chronic disease.

INTRODUCTION

Anemia has been recognized as one of the most significant burdens on health care worldwide and, although common among surgical patients, it is rarely treated preoperatively, as it is perceived as a mere consequence of aging or a marker of disease.[1-3] Due to the delicate balance between either the sequelae of tissue hypoxia or thrombosis, as well as the complex interplay between known contributors to worse surgical outcomes – in particular the triad of anemia, blood loss, and transfusions – the risk profile of vascular patients appears to be more profound. Patients with chronic limb-threatening ischemia (CLTI) may be at even higher risk, not only from the inherent surgical complexity of revascularization but also from the high proportion of elderly patients and comorbid conditions.

Blood transfusion continues to be the mainstay therapy of anemia in surgical patients, although a significant percentage of transfusions to hospitalized patients have been identified as inappropriate.[4,5] Minimization or avoidance of transfusions has gained more acceptance in the last few years as more data emerge about the risks.[6-8] A multitude of different medical societies recommend restrictive transfusion regimens, with a hemoglobin trigger of 7 – 8 g/dL, as long as there are no signs of end-organ ischemia.[9-11] These clear guidelines are not necessarily implemented in daily clinical practice and, surprisingly, there are no specific practice guidelines from vascular societies with regard to blood product usage or management of preoperative anemia. Studies in peripheral arterial disease have repeatedly suggested that both anemia and transfusion are independently associated with higher mortality and morbidity.[12-21] Importantly, these studies did not address the interplay between these two risk factors and failed to appropriately adjust for one another. Moreover, long-term outcomes remain uncertain.

To better comprehend the triad of anemia, blood loss, and transfusion in patients with CLTI, we investigated periprocedural as well as long-term outcomes in those with preoperative anemia, and determined whether these outcomes varied by blood transfusion.

METHODS

Registry and study population

A retrospective cohort study was performed of patients with CLTI who subsequently underwent infrainguinal bypass surgery at one of the 31 medical centers participating in the Vascular Study Group of New England (VSGNE) registry from 2008 to 2015. VSGNE is a regional collaboration of academic and community hospitals started by vascular surgeons, which prospectively collects data on vascular procedures with the aim of improving quality of surgical and patient care. More than 140 detailed clinical variables, including 1-year follow-up outcomes, are gathered and entered into the registry by trained nurses and clinical abstractors. Regional quality assurance and validation have been previously described[22] and additional information is available at: http://www.vascularqualityinitiative.org/

components-of-the-vqi/regional-quality-groups/current-regional-quality-groups/vascular-study-group-of-new-england/. All patient data from the VSGNE registry are de-identified and, therefore, approval was obtained from the Institutional Review Board of the Beth Israel Deaconess Medical Center and informed consent was waived.

We included patients with CLTI undergoing infrainguinal bypass starting in 2008, as previous years lacked hemoglobin data. Emergent cases (N = 33, 1.1%) and patients missing crucial variables in regard to hemoglobin, blood loss, or transfusion (N = 83, 2.9%) were additionally excluded. Preoperative hemoglobin concentration was collected by VSGNE and defined as the last value obtained before entry to the operating room. Following the World Health Organization (WHO) recommendations, female patients with a preoperative hemoglobin concentration of < 12 g/dL and male patients with a preoperative hemoglobin concentration of < 13 g/dL were classified as anemic.[23] The study cohort was subsequently stratified by hemoglobin concentration into three arbitrary groups: moderate/severe anemia (≤ 9 g/dL), mild anemia (male: 9.1 - 12.9 g/dL, female: 9.1 - 11.9 g/dL), and no anemia (male: ≥ 13 g/dL, female: ≥ 12 g/dL). As less than 2% of our study population had a hemoglobin concentration of ≤ 8 g/dL, we were unable to construct an additional severe only anemia group.

Study variables

Among preoperative variables, end-stage renal disease was classified as either a functioning transplant or preoperative dialysis. Estimated intraoperative blood loss was captured and presented as both a continuous and categorical variable. Patients in the upper 80th percentile (≥ 400 mL) were defined as those with more excessive blood loss, which allowed us to stratify our cohort and maintain a sufficient number to adjust properly.

The primary endpoints of this study were in-hospital cardiac complications, one-year mortality of any cause, and the composite outcome of one-year major amputation (defined as below or above knee level) or death. Cardiac complications were considered when one of the following was documented: new myocardial infarction, any new rhythm disturbances requiring medication or cardioversion, or postoperative diagnosis of congestive heart failure (defined as pulmonary edema with monitoring or treatment in ICU). Secondary outcome measures were recorded and included in-hospital (i.e., blood transfusion, renal deterioration, return to the operating room, bleeding, graft thrombosis, surgical site infection, discharge not to home, length of stay), 30-day (i.e., major amputation, bypass revision), and one-year adverse events (i.e., major adverse limb event [MALE]). Deterioration of renal function was defined as an increase of postoperative serum creatinine of 0.5 mg/dL and/or the need for new dialysis. Blood transfusion was defined by VSGNE as any transfusion of packed red blood cells during hospitalization, which included preoperative, intraoperative, and postoperative transfusion. Neither the timing nor the indication of transfusion were captured by VSGNE. MALE included an ipsilateral major amputation or the need for a secondary major intervention of the index limb, such as a new, jump or interposition graft, thrombectomy, or thrombolysis.[24]

Statistical analysis

Our study consisted of two main analyses. First, differences in patient characteristics and outcomes were compared across the levels of anemia using existing data elements of the VSGNE. All P-values are relative to our reference group: no anemia. To avoid repeated measures and confounding due to within-patient dependence, only the initial bypass procedure of each patient recorded in the VSGNE was considered. Pearson's χ^2 and Fisher's exact test, as well as the Student's t-test and Mann Whitney U test were performed as appropriate, and values are accordingly provided in the form of percentages, mean ± standard deviation, or median with interquartile range. Multivariable logistic regression was performed to determine associations between anemia and in-hospital or 30-day outcomes, whereas Cox proportional hazard models were used for one-year follow-up outcomes. For all multivariable analyses, we included covariates that were deemed clinically relevant as well as characteristics identified by univariate screen ($P < .10$), using the purposeful selection of variables method.[25]

As previously described, we hypothesize that one mechanism by which preoperative anemia contributes to the increased risk of adverse events is through an important intermediate event, perioperative blood transfusions. This is supported by the fact that preoperative anemia is one of the strongest predictors of transfusion.[19,21] As such, controlling for intermediate variables may be viewed as a form of overadjustment. Since this would bias results to the null hypothesis, the direct effect of our exposure variable (anemia) cannot be consistently estimated. As a result, we did not control for blood transfusions in our initial multivariable models, which allowed for more comprehensive predictions. Because we were still interested in whether the associations between preoperative anemia and adverse outcomes varied by blood transfusions, we included interaction terms between anemia categories and blood transfusion in a secondary analysis. For statistical calculations, SPSS Statistics 23 (IBM Corp, Armonk, NY) was used with $P < .05$ considered to be statistically significant.

RESULTS

Over the eight-year study period, 2,822 patients with CLTI underwent an infrainguinal bypass and of these patients, 219 (7.8%) presented with moderate/severe anemia, 1,376 (49%) with mild anemia, and 1,227 (43%) without preoperative anemia.

Baseline and operative characteristics

Table I provides the baseline characteristics. Compared to patients without preoperative anemia, those with anemia were older, less likely white or smokers (prior or current), and more likely in need of assistance with ambulation. Anemic patients more often presented with comorbid conditions, such as hypertension, coronary artery disease, history of coronary interventions, congestive heart failure, diabetes, and end-stage renal disease (all $P < .001$).

Table I. Baseline characteristics for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass

	Moderate/ severe anemia (N=219)		P-value vs. no anemia	Mild anemia (N=1376)		P-value vs. no anemia	No anemia (N=1227)	
	N	*%*		*N*	*%*		*N*	*%*
Age (years, mean ± SD)	69.9 (11)		**<.001**	70.2 (11)		**<.001**	66.0 (12)	
Male gender	111	(51)	**<.001**	929	(68)	.23	801	(65)
White	166	(76)	**<.001**	1209	(88)	**<.001**	1132	(92)
Obesity (BMI > 30)	76	(35)	.31	370	(27)	**<.01**	387	(32)
Smoking			**<.001**			**<.001**		
Never	50	(23)		289	(21)		170	(14)
Prior	115	(52)		683	(50)		444	(36)
Current	54	(25)		402	(29)		613	(50)
Hypertension	204	(93)	**<.001**	1257	(91)	**<.001**	1029	(84)
Coronary artery disease	88	(40)	**<.001**	513	(37)	**<.001**	336	(27)
Prior CABG/PCI	82	(38)	**<.001**	494	(36)	**<.001**	297	(24)
Congestive heart failure	71	(32)	**<.001**	339	(25)	**<.001**	130	(11)
Diabetes	153	(70)	**<.001**	889	(65)	**<.001**	547	(45)
COPD	56	(26)	.57	328	(24)	.97	292	(24)
End-stage renal disease	41	(19)	**<.001**	153	(11)	**<.001**	29	(2.4)
Pre-op ambulatory status			**<.001**			**<.001**		
Ambulatory	117	(53)		929	(68)		1025	(84)
Ambulatory with Assistance	78	(36)		364	(27)		173	(14)
Wheelchair	19	(8.7)		72	(5.2)		28	(2.3)
Bedridden	5	(2.3)		10	(0.7)		1	(0.1)
Symptom status			**<.001**			**<.001**		
Rest pain	35	(16)		344	(25)		632	(51)
Tissue loss	184	(84)		1032	(75)		595	(49)
Prior lower extremity procedures								
Open bypass	33	(15)	.10	148	(11)	.74	137	(11)
Endovascular intervention	37	(17)	.06	192	(14)	.19	150	(12)
Pre-op medications								
Aspirin	168	(77)	.40	1067	(78)	.30	972	(79)
P2Y12 antagonist	45	(21)	.06	245	245	.11	190	(16)
Anticoagulation [51%]	28	(21)	**.045**	123	123	.05	82	(14)
Statin	150	(69)	.26	1054	1054	**.01**	886	(72)
Hemoglobin concentration (mean ± SD)	8.3	(0.7)	**<.001**	11.1	11.1	**<.001**	14.1	(1.3)

SD: standard deviation, *BMI*: body mass index, *CABG*: coronary artery bypass graft, *PCI*: percutaneous coronary intervention, *COPD*: chronic obstructive pulmonary disease.
[ ] indicates percentage data missing, only variables with >5% missing are reported.

In addition, the proportion of tissue loss was higher among patients with lower hemoglobin concentrations (moderate/severe: 84%, mild: 75%, and no anemia: 49%; all P < .001). The mean preoperative hemoglobin concentration was 8.3 ± 0.7 g/dL in patients with moderate/severe anemia, 11.1 ± 0.7 in those with mild anemia, and 14.1 ± 1.3 in those without anemia (P < .001).

Operative details are reported in Table II. The popliteal artery was more commonly utilized as an inflow artery among anemic patients, whereas the femoral artery was less frequently used compared to those without anemia. Additionally, the targets for revascularization were more likely distal for those with preoperative anemia. Patients with lower hemoglobin concentrations on admission were less often revascularized with saphenous veins compared to those without anemia. Intraoperative blood loss was higher in those with moderate/severe or mild anemia (315 ± 273 and 293 ± 315 mL, respectively) compared to non-anemic patients (263 ± 294 mL; all P < .05). There were no differences in concomitant procedures, including endovascular interventions and endarterectomy. Finally, anemic patients were less likely to undergo an elective compared to urgent procedure and the total operative time was notably longer compared to those with normal hemoglobin concentrations on admission.

Perioperative outcomes

Following a bypass procedure for CLTI, total length of hospital stay was significantly longer among patients with preoperative anemia compared to those without anemia (moderate/severe: 6 days [IQR: 4-10], mild: 5 [3-7], and no anemia: 4 [3-6]; all P < .001) (Table III). On univariate analysis, in-hospital cardiac complications were higher among patients with anemia (moderate/severe: 12%, mild: 11%, and no anemia: 6.6%; all P < .01). Preoperative anemia was further associated with higher rates of renal deterioration and return to the operating room during the index hospitalization. Blood transfusions were significantly more frequent among anemic patients (moderate/severe: 70%, mild: 36%, and no anemia: 12%; all P < .001), with simultaneously higher numbers of units transfused in those with lower hemoglobin levels (moderate/severe: 2.6 ± 2.7 units, mild: 1.0 ± 2.3, and no anemia: 0.4 ± 1.5; all P < .001). Rates of mortality within 30 days increased as preoperative hemoglobin concentration decreased (moderate/severe: 4.6%, mild: 3.6%, no anemia: 1.3%, all P < .01). Patients with moderate/severe anemia were also more likely to undergo a major amputation during the first 30 days compared to non-anemic patients (5.0% vs. 1.4%, P < .001).

After adjustment for key confounders, preoperative anemia was not significantly associated with in-hospital cardiac complications (moderate/severe: odds ratio (OR) 1.27, [95% confidence interval CI 0.77 – 2.10], mild: OR 1.29 [0.95 – 1.74]), nor with mortality within 30 days (moderate/severe: OR 1.90 [0.81 – 4.45], mild: OR 1.82 [0.99 – 3.33]) compared to no anemia (Table V).

Table II. Procedure details for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass

	Moderate/ severe anemia *(N=219)*		**P-value vs. no anemia**	**Mild anemia** *(N=1376)*		**P-value vs. no anemia**	**No anemia** *(N=1227)*	
	N	*%*		*N*	*%*		*N*	*%*
ASA Class > 3 [7.5%]	47	(23)	**<.001**	258	(20)	**<.001**	111	(9.9)
Elective (vs. Urgent)	131	(60)	**<.001**	909	(66)	**<.001**	950	(77)
Graft origin			**<.001**			**<.01**		
Femoral	172	(79)		1190	(87)		1110	(91)
Popliteal	45	(21)		178	(13)		108	(8.8)
Tibial	2	(0.9)		3	(0.2)		6	(0.5)
Graft recipient			**<.001**			**<.001**		
Femoral	3	(1.4)		14	(1.0)		23	(1.9)
Popliteal	80	(37)		593	(43)		666	(55)
Tibial	95	(44)		563	(41)		435	(36)
Pedal	39	(18)		201	(15)		96	(7.9)
Conduit type			**<.01**			.32		
Saphenous vein	126	(58)		917	(67)		848	(70)
Prosthetic	66	(30)		336	(25)		289	(24)
Arm vein	8	(3.7)		28	(2.0)		20	(1.6)
Composite vein	10	(4.6)		52	(3.8)		41	(3.4)
Composite vein and prosthetic	8	(3.7)		39	(2.8)		22	(1.8)
Intraoperative blood loss			**<.001**			**<.01**		
< 400 mL	154	(70)		1052	(76)		995	(81)
≥ 400 mL	65	(30)		324	(24)		232	(19)
Intraoperative blood loss (mL, mean ± SD)	315 (273)		**.02**	293 (315)		**.01**	263 (294)	
Concomitant procedures								
Endovascular [6.0%]	13	(6.2)	.37	88	(6.8)	.25	92	(8.0)
Endarterectomy [6.6%]	51	(24)	.22	379	(29)	.64	325	(29)
Procedure time (min, median ± IQR) [19%]	242 (185-340)		**.02**	242 (185-316)		**.01**	233 (179-302)	

ASA: American Society of Anesthesiologists, *SD*: standard deviation, min: minutes, *IQR*: interquartile range.
[ ] indicates percentage data missing, only variables with >5% missing are reported.

Table III. Perioperative outcomes for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass

	Moderate/ severe anemia *(N=219)*		**P-value vs. no anemia**	**Mild anemia** *(N=1376)*		**P-value vs. no anemia**	**No anemia** *(N=1227)*	
	N	*%*		*N*	*%*		*N*	*%*
In-hospital outcomes								
Mortality	5	(2.3)	**.02**	26	(1.9)	**<.01**	8	(0.7)
Cardiac complication	26	(12)	**<.01**	153	(11)	**<.001**	81	(6.6)
Myocardial infarction	11	(5.0)	.06	70	(5.1)	**<.01**	33	(2.7)
Dysrhythmia	10	(4.6)	.48	82	(6.0)	**<.01**	44	(3.6)
Congestive heart failure	12	(5.5)	**<.01**	51	(3.7)	**<.01**	24	(2.0)
Blood transfusion	154	(70)	**<.001**	498	(36)	**<.001**	147	(12)
Units of PRBC (mean ± SD)	2.6	(2.7)	**<.001**	1.0	(2.3)	**<.001**	0.4	(1.5)
Renal deterioration	17	(7.8)	**<.001**	90	(6.6)	**<.001**	30	(2.5)
Return OR	58	(27)	**<.001**	191	(14)	**<.01**	121	(9.9)
Bleeding	7	(3.2)	**<.01**	9	(0.7)	.48	11	(0.9)
Graft thrombosis	9	(4.1)	.89	27	(2.0)	**<.01**	48	(3.9)
Surgical site infection	10	(4.6)	.65	61	(4.4)	.51	48	(3.9)
Discharge not to home	151	(69)	**<.001**	754	(55)	**<.001**	386	(32)
Length of hospital stay (days, median ± IQR)	6 (4-10)		**<.001**	5 (3-7)		**<.001**	4 (3-6)	
30-day outcomes								
Mortality	10	(4.6)	**.001**	50	(3.6)	**<.001**	16	(1.3)
Major amputation	11	(5.0)	**<.001**	21	(1.5)	.76	17	(1.4)
Bypass revision	4	(1.8)	.64	25	(1.8)	.14	33	(2.7)

PRBC: Packed red blood cells, *OR:* operating room, *SD*: standard deviation, *IQR*: interquartile range.

One-year outcomes

In crude analysis at one year after bypass, mortality was substantially higher among patients with preoperative anemia and declined with increasing hemoglobin concentrations (moderate/severe: 21%, mild: 17%, and no anemia: 6.5%; all P < .001) (Table IV). Further, patients with preoperative anemia also had higher rates of the composite outcome, one-year major amputation or death (moderate/severe: 32%, mild: 23%, and no anemia: 12%; all P < .001), while major amputation was only more common in patients with moderate/severe compared to no anemia (14% vs. 5.5%, P < .001). Rates of MALE were similar between anemic and non-anemic patients.

Utilizing multivariable regression, preoperative anemia was significantly associated with higher one-year mortality for moderate/severe (hazard ratio (HR) 2.17, [95% CI 1.48 – 3.20]) and mild anemia (HR 1.87 [1.43 – 2.45]) (Table V). Furthermore, both moderate/severe and mild anemia were independently associated with the combined outcome of one-year major amputation or death (HR 2.00 [1.48 – 2.71] and HR 1.48 [1.20 – 1.83], respectively), while only moderate/severe anemia was associated with a higher rate of major amputation (HR 2.25 [1.42 – 3.57]) compared to those without anemia.

Interactions between anemia and blood transfusion

In a secondary analysis, we evaluated trends of our primary outcomes in patients with and without blood transfusions across the different categories of anemia in the overall study cohort and stratified by age (≤ 75 vs. > 75 years), as well as by operative blood loss (< 400 vs. ≥ 400 mL). As detailed in Figure 1, the risk of mortality at one year was not affected by blood transfusions in patients with preoperative anemia, whereas in those without anemia, transfusions were associated with significantly higher mortality compared to no transfusion.

Table IV. Unadjusted outcomes at one-year follow-up for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass

	Moderate/ severe anemia *(N=219)*		**P-value vs. no anemia**	**Mild anemia** *(N=1376)*		**P-value vs. no anemia**	**No anemia** *(N=1227)*	
	N	*%*		*N*	*%*		*N*	*%*
One-year outcomes								
Mortality	45	(21)	**<.001**	230	(17)	**<.001**	80	(6.5)
Major amputation or death	69	(32)	**<.001**	312	(23)	**<.001**	144	(12)
Major amputation	30	(14)	**<.001**	92	(6.7)	.22	68	(5.5)
MALE	44	(20)	.70	245	(18)	.47	233	(19)

MALE: major adverse limb event

Table V. Multivariable analyses for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass with the reference group no anemia (blood transfusion not added as a covariate)

	Moderate/severe Anemia			**Mild Anemia**		
	OR	*[95% CI]*	*P-value*	*OR*	*[95% CI]*	*P-value*
In-hospital cardiac complication[a]	1.27	[0.77 – 2.10]	.34	1.29	[0.95 – 1.74]	.10
30-day mortality[b]	1.90	[0.81 – 4.45]	.14	1.82	[0.99 – 3.33]	.05
	HR	*[95% CI]*	*P-value*	*HR*	*[95% CI]*	*P-value*
One-year mortality[b]	2.17	[1.48– 3.20]	**<.001**	1.87	[1.43 – 2.45]	**<.001**
One-year major amp or death[b]	2.00	[1.48 – 2.71]	**<.001**	1.48	[1.20 – 1.83]	**<.001**
One-year major amputation[b]	2.25	[1.42 – 3.57]	**.001**	1.11	[0.79 – 1.55]	.55

OR: odds ratio, *CI*: confidence interval, *HR*: hazard ratio. [a] Adjusted for: age, gender, symptom status, race, coronary artery disease, history of CABG/PCI, diabetes, end-stage renal disease, COPD, intraoperative blood loss. [b] Adjusted for: age, gender, symptom status, race, coronary artery disease, diabetes, end-stage renal disease, COPD, intraoperative blood loss, conduit type

This association was specifically observed in non-anemic patients who were older than 75 years (HR 2.97 [1.35 – 6.55]) and in those with operative blood loss of less than 400 mL (HR 2.77 [1.40 – 5.48]). Of note, the unadjusted mortality rates among patients above 75 years with moderate/severe anemia were approximately 2.5 times higher than their younger counterparts (≤ 75 years) for those transfused (35.8% vs. 13.9%) and not transfused (35.3% vs. 12.5%). In addition, among patients with more excessive blood loss (≥ 400 mL), we found a hazard ratio of less than 1 for one-year mortality (HR 0.77 [0.20 – 2.90]) in patients with moderate/severe anemia who received blood transfusions, although statistical significance was not reached.

Furthermore, transfusions were associated with significantly higher cardiac complications during index hospitalization in patients with moderate/severe (OR 3.76 [1.07 – 13.22]), mild (OR 2.59 [1.79 – 3.76]), and no preoperative anemia (OR 5.17 [3.09 – 8.64]) compared to those not receiving any blood transfusions (Figure 2). These associations were consistent among patients ≤ 75 and > 75 years, as well as in those with operative blood loss of < 400 and ≥ 400 mL. A similar analysis for the composite endpoint major amputation or death at one year is presented as Supplementary Figure 1.

Figure 1. One-year mortality for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass with an interaction term between anemia and blood transfusions included

	One-year Mortality / Total No. (%)					
	Transfusion		**No Transfusion**		**Adjusted HR [95% CI]**	
Overall (N=2822)						
Moderate/severe anemia	33/154	(21.4)	12/65	(18.5)	1.51	[0.77 - 2.95]
Mild anemia	94/498	(18.9)	136/877	(15.5)	1.18	[0.89 - 1.55]
No anemia	18/147	(12.2)	62/1080	(5.7)	**1.79**	[1.04 - 3.10]
Age ≤ 75 years (N=1980)						
Moderate/severe anemia	14/101	(13.9)	6/48	(12.5)	1.44	[0.55 - 3.80]
Mild anemia	48/306	(15.7)	69/794	(12.0)	1.28	[0.87 - 1.88]
No anemia	8/106	(7.5)	43/845	(5.1)	1.15	[0.53 - 2.53]
Age > 75 years (N=842)						
Moderate/severe anemia	19/53	(35.8)	6/17	(35.3)	1.36	[0.52 - 3.50]
Mild anemia	46/192	(24.0)	67/304	(22.0)	1.04	[0.69 - 1.56]
No anemia	10/41	(24.4)	19/235	(8.1)	**2.97**	[1.35 - 6.55]
Blood loss < 400 mL (N=2201)						
Moderate/severe anemia	22/237	(21.8)	9/53	(17.0)	1.76	[0.80 - 3.87]
Mild anemia	49/280	(17.5)	124/772	(16.1)	1.05	[0.75 - 1.47]
No anemia	10/63	(15.9)	53/932	(5.7)	**2.77**	[1.40 - 5.48]
Blood loss ≥ 400 mL (N=621)						
Moderate/severe anemia	11/53	(20.8)	3/12	(25.0)	0.77	[0.20 - 2.90]
Mild anemia	45/218	(20.6)	12/105	(11.4)	1.71	[0.90 - 3.26]
No anemia	8/84	(9.5)	9/148	(6.1)	0.95	[0.36 – 2.50]

Adjusted for: age, gender, symptom status, race, coronary artery disease, diabetes, end-stage renal disease, COPD, intraoperative blood loss, conduit type. *HR*: hazard ratio, *CI*: confidence interval

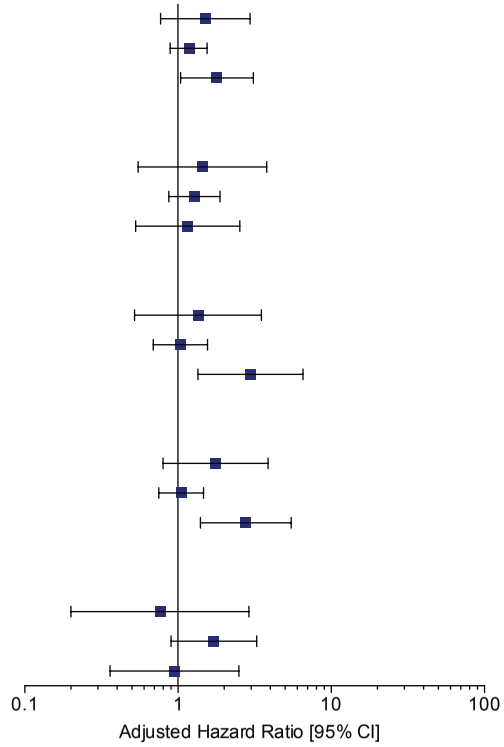
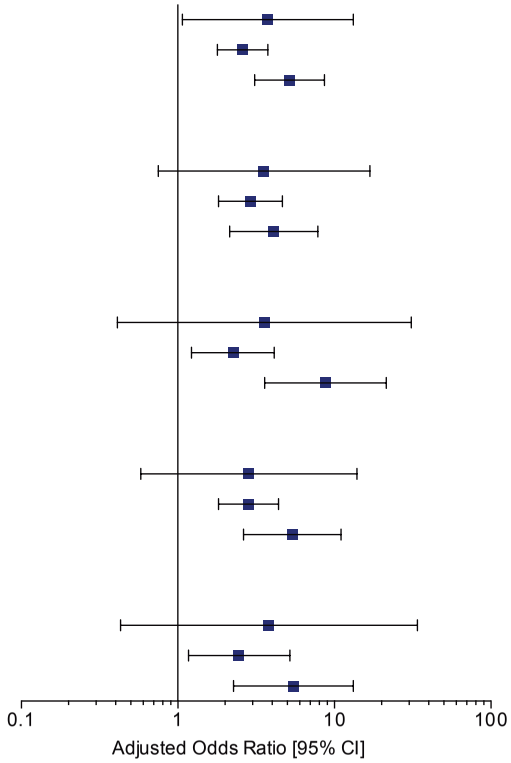


Figure 2. In-hospital cardiac complication for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass with an interaction term between anemia and blood transfusions included

	In-hospital cardiac complication / Total No. (%)					
	Transfusion		**No Transfusion**		**Adjusted OR [95% CI]**	
Overall (N=2822)						
Moderate/severe anemia	23/153	(15.0)	3/65	(4.6)	**3.76**	[1.07 - 13.22]
Mild anemia	92/497	(18.5)	61/877	(7.0)	**2.59**	[1.79 - 3.76]
No anemia	36/147	(24.5)	45/1079	(4.2)	**5.17**	[3.09 - 8.64]
Age ≤ 75 years (N=1980)						
Moderate/severe anemia	13/101	(12.9)	2/48	(4.2)	3.54	[0.75 - 16.83]
Mild anemia	58/306	(19.0)	36/574	(6.3)	**2.91**	[1.82 - 4.66]
No anemia	20/106	(18.9)	32/845	(3.8)	**4.10**	[2.14 - 7.85]
Age > 75 years (N=842)						
Moderate/severe anemia	10/52	(19.2)	1/17	(5.9)	3.58	[0.41 - 31.01]
Mild anemia	34/191	(17.8)	25/303	(8.3)	**2.25**	[1.22 - 4.13]
No anemia	16/41	(39.0)	13/234	(5.6)	**8.78**	[3.59 - 21.48]
Blood loss < 400 mL (N=2201)						
Moderate/severe anemia	9/101	(8.9)	2/53	(3.8)	2.85	[0.58 - 13.93]
Mild anemia	45/280	(16.1)	50/772	(6.5)	**2.82**	[1.82 - 4.39]
No anemia	13/63	(20.6)	37/931	(4.0)	**5.38**	[2.63 - 11.02]
Blood loss ≥ 400 mL (N=612)						
Moderate/severe anemia	14/52	(26.9)	1/12	(8.3)	3.80	[0.43 - 33.85]
Mild anemia	47/217	(21.7)	11/105	(10.5)	**2.46**	[1.17 - 5.20]
No anemia	23/84	(27.4)	8/148	(5.4)	**5.47**	[2.27 - 13.21]

Adjusted for: age, gender, symptom status, race, coronary artery disease, history of CABG/PCI, diabetes, end-stage renal disease, COPD, intraoperative blood loss. *OR*: odds ratio, *CI*: confidence interval



DISCUSSION

This study indicates that among CLTI patients undergoing a bypass procedure more than half present with anemia on admission. Notably, late – but not early – mortality increased as preoperative hemoglobin levels decreased, and similar associations were observed for one-year major amputation. Cardiac complications during hospitalization were not affected by preoperative anemia; however, these data demonstrated that early cardiac adverse events were associated with higher transfusion rates in those with and without anemia. In addition, receiving blood transfusion at normal hemoglobin concentrations was associated with higher mortality compared to those without a transfusion, and as hemoglobin decreased, we found no significant association between blood transfusion and the rate of mortality in anemic patients, which was irrespective of age or operative blood loss.

Our findings enhance existing literature evaluating anemia in more advanced stages of peripheral arterial disease. While the overall prevalence of anemia in the general population of 65 years and older is approximately 10%,[26-28] this number is strikingly higher for CLTI. In our regional study, 57% of patients undergoing bypass were anemic, which is in line with prior reports documenting a range of 49% to 75%.[12-15] Even though anemia is highly prevalent in this patient population, the significance of preoperative anemia appears to be underappreciated and its risks routinely overlooked. As in other surgical specialties, patients with preoperative anemia undergoing invasive procedures have a higher risk of early and late adverse outcomes.[2,3,29-33] Ultimately, hemoglobin concentration is not simply a laboratory value but rather a modifiable risk factor, and interpretation as well as correction before surgery may be the key to successful management and to achieve favorable patient outcomes. Measures and efforts to devise best practices have been bundled in a multidisciplinary concept, known as "patient blood management".[34-36] One of the three pillars discusses anemia management with iron and/or erythropoietin supplementation, which aims to change care paradigms from the perspective of transfusion as a therapy to addressing the underlying clinical condition, even for anemia of chronic disease.

Few studies reported on long-term outcomes among anemic patients undergoing a peripheral intervention, although none adjusted or stratified for blood loss or transfusion. Desormais et al. studied a subset of 510 patients with CLTI undergoing revascularization in a French multicenter registry and found that preoperative anemia (in accordance to WHO guidelines) was associated with the combined outcome amputation or death at one year (HR 1.14 [1.03 – 1.26]) as well as major amputation alone (HR 1.13 [1.03 – 1.25]).[12] While the previous study did not report mortality separately, a single-institution study of 403 patients with CLTI demonstrated that preoperative anemia – defined as hemoglobin concentration < 10 g/dL – was associated with worse long-term survival (HR 2.5 [1.8 – 3.4]).[13] Importantly, these studies did not distinguish between severities of anemia, which, as our data showed, may provide more detailed risk stratification in respect to long-term outcomes. In particular, moderate/severe anemia was associated with a 2.3-fold higher risk of major amputation, whereas a milder degree of anemia was not associated with limb events.

In contrast to long-term outcomes, multiple studies have elaborated on 30-day adverse events with conflicting results. In 2013, a study conducted among 31,857 patients admitted for various non-cardiac elective vascular procedures demonstrated that intraoperative blood transfusions and decreasing hemoglobin levels on admission were inversely associated with major adverse cardiovascular events within 30 days (defined as myocardial infarction or death).[16] Conversely, Kougias et al. demonstrated that, among 1,074 vascular procedures, the units of packed red blood cells transfused – but not anemia – were independently associated with acute coronary syndrome (OR 1.13 [1.01 – 1.27]).[18] Although VSGNE only captures in-hospital cardiac outcomes, our study also found no correlation between adverse cardiac events and preoperative anemia in CLTI patients. Yet, instead we demonstrated a significant association between blood transfusion and cardiac complications regardless of hemoglobin concentration. These data may further corroborate previous literature and may indicate that blood product usage in patients with mild to moderate anemia possibly predisposes a thrombotic or ischemic event, and increases rather than decreases the risk of coronary adverse outcomes.

Given that operative blood loss influences the decision to transfuse patients, we attempted to account for this by stratifying our cohort. Similarly, Wu et al. analyzed a heterogeneous cohort of 239,286 elderly patients undergoing major non-cardiac surgery and concluded that blood transfusions were associated with lower mortality in all patients with preoperative hematocrit levels of less than 24% and in those with mild or no preoperative anemia who experienced more excessive blood loss of 500 – 999 mL.[37] In contrast, higher mortality rates were found in transfused patients without excessive blood loss (< 500 mL) and hematocrit levels of 30% or higher. Consistent with this rationale, the current study found similar results, albeit limited by our sample size. Nonetheless, our study supports the notion of avoiding transfusions in patients with milder degrees of anemia and minimal operative blood loss.

Supported by growing evidence, more and more medical professional organizations have issued evidence-based practice guidelines that reflect restrictive transfusion protocols, including the American Association of Blood Banks.[11] An important limitation of the current study is that hemoglobin levels at the time of transfusion and specific indications were not captured by VSGNE. Despite this inherent limitation, approximately 20% of our study cohort, which consisted primarily of patients with preoperative hemoglobin concentrations of greater than 8 g/dL, was transfused in the perioperative setting without excessive intraoperative blood loss. Although our data do not allow us to comment on transfusion thresholds, it merely presents an opportunity to reduce avoidable risks of current transfusion practices, but it requires significant effort to translate evidence from well-conducted studies into routine clinical care.

Several limitations to this study merit consideration. Although all data were collected prospectively, an observational study design, like ours, is obviously prone to residual and unmeasured confounding as all variables are predetermined by VSGNE and non-modifiable. Moreover, as previously motioned, we could not evaluate the exact clinical indication of

transfusion nor were we able to determine the hemoglobin concentration at the time of transfusion, which could have confounded our results. Since the safety of restrictive transfusion strategies has been demonstrated in the last few years, physicians may be more adapted to these protocols today, whereas our study dates back to 2008. Hemoglobin levels during the time of follow-up were additionally not captured by VSGNE. Furthermore, while our study illustrates regional practices in the United States, generalizability to the population at large may be hindered by the overrepresentation of whites and exclusively analyzing patients who underwent bypass. Finally, blood transfusion could have taken place either before, during, or after the bypass procedure and, unfortunately, this is not delineated further with VSGNE data. This is particularly interesting in distinguishing whether the cardiac events were prior or post blood transfusions.

CONCLUSION

This study highlights the higher risk of late mortality and limb events in CLTI patients with preoperative anemia who undergo bypass, with the highest event rates in those with the lowest hemoglobin levels. Blood transfusions were strongly correlated with anemia and increased - rather than decreased - in-hospital and late postoperative adverse outcomes in selected patients. As such, the appropriateness of excessive blood product usage as well as acceptable hemoglobin targets should be reevaluated in this patient population. Both acknowledging its risks and creating awareness of alternative treatment approaches may benefit these burdened patients throughout the course of care.

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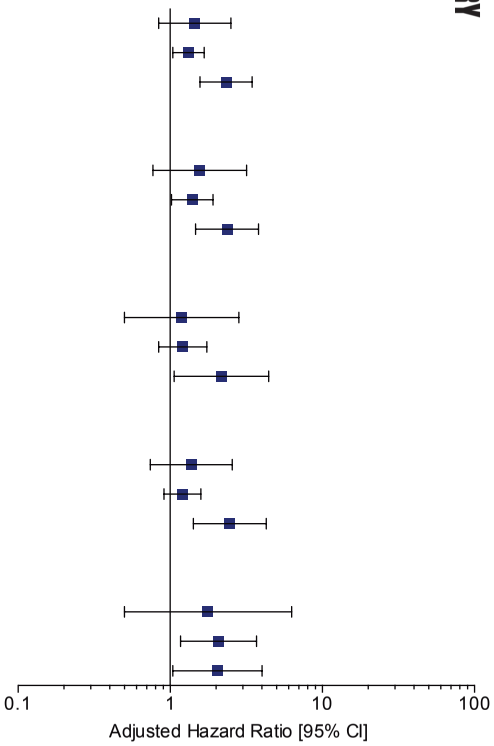
Supplementary figure 1. One-year major amputation or death for patients with chronic limb-threatening ischemia (CLTI) undergoing bypass with an interaction term between anemia and blood transfusions included

	One-year major amp or death / Total No. (%)					
	Transfusion		**No Transfusion**		**Adjusted HR [95% CI]**	
Overall (N=2822)						
Moderate/severe anemia	51/154	(33.1)	18/65	(27.7)	1.45	[0.84 - 2.51]
Mild anemia	132/496	(26.6)	180/877	(20.5)	**1.32**	[1.04 - 1.67]
No anemia	37/147	(25.2)	107/1080	(9.9)	**2.33**	[1.57 - 3.46]
Aged ≤ 75 years (N=1980)						
Moderate/severe anemia	29/101	(28.7)	11/48	(22.9)	1.57	[0.77 - 3.18]
Mild anemia	74/306	(24.2)	100/573	(17.5)	**1.39**	[1.02 - 1.91]
No anemia	26/106	(24.5)	78/845	(9.2)	**2.37**	[1.47 - 3.81]
Aged > 75 years (N=842)						
Moderate/severe anemia	22/53	(41.5)	7/17	(41.2)	1.19	[0.50 - 2.83]
Mild anemia	58/190	(30.5)	80/304	(26.3)	1.21	[0.84 - 1.74]
No anemia	11/41	(26.8)	29/235	(12.3)	**2.17**	[1.06 - 4.44]
Blood loss < 400 mL (N=2201)						
Moderate/severe anemia	33/101	(32.7)	15/53	(28.3)	1.38	[0.74 - 2.56]
Mild anemia	72/280	(25.7)	165/772	(21.4)	1.20	[0.91 - 1.59]
No anemia	15/63	(23.8)	92/923	(9.9)	**2.46**	[1.42 - 4.28]
Blood loss ≥ 400 mL (N=621)						
Moderate/severe anemia	18/53	(34.0)	3/12	(25.0)	1.77	[0.50 - 6.28]
Mild anemia	60/216	(27.8)	15/105	(14.3)	**2.08**	[1.17 - 3.69]
No anemia	22/84	(26.2)	15/148	(10.1)	**2.04**	[1.04 - 4.01]

Adjusted for: age, gender, symptom status, race, coronary artery disease, diabetes, end-stage renal disease, COPD, intraoperative blood loss, conduit type. *HR*: hazard ratio, *CI*: confidence interval



CHAPTER 7

RISK FACTORS FOR 30-DAY UNPLANNED READMISSIONS FOLLOWING INFRAINGUINAL ENDOVASCULAR INTERVENTIONS

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ABSTRACT

Objective

Unplanned hospital readmissions following surgical interventions are associated with adverse events and contribute to increasing healthcare costs. Despite numerous studies defining risk factors following lower extremity bypass surgery, evidence regarding readmission after endovascular interventions is limited. This study aims to identify predictors of 30-day unplanned readmission following infrainguinal endovascular interventions.

Methods

We identified all patients undergoing an infrainguinal endovascular intervention in the Targeted Vascular module of the American College of Surgeons National Surgical Quality Improvement Program between 2012 and 2014. Perioperative outcomes were stratified by symptom status (chronic limb-threatening ischemia [CLTI] vs. claudication). Patients who died during index admission, and those who remained in the hospital after 30 days, were excluded. Indications for unplanned readmission related to the index procedure were evaluated. Multivariable logistic regression was used to identify preoperative and in-hospital (during index admission) risk factors of 30-day unplanned readmission.

Results

4,449 patients underwent infrainguinal endovascular intervention, of which 2,802 (63%) had CLTI (66% tissue loss) and 1,647 (37%) had claudication. The unplanned readmission rates for CLTI and claudication patients were 16% (N=447) and 6.5% (N=107), respectively. Mortality after index admission was higher for readmitted patients compared to those not readmitted (CLTI: 3.4% vs. 0.7%, $P < .001$; claudication: 2.8% vs. 0.1%, $P < .01$). Approximately 50% of all unplanned readmissions were related to the index procedure. Among CLTI patients, the most common indication for readmission related to the index procedure was wound- or infection-related (42%), while patients with claudication were mainly readmitted for recurrent symptoms of peripheral vascular disease (28%). In patients with CLTI, predictors of unplanned readmission included diabetes (OR: 1.3, 95% CI: 1.01-1.6), congestive heart failure (1.6, 1.1-2.5), renal insufficiency (1.7, 1.3-2.2), preoperative dialysis (1.4, 1.02-1.9), tibial angioplasty/stenting (1.3, 1.04-1.6), in-hospital bleeding (1.9, 1.04-3.5), in-hospital unplanned return to the operating room (1.9, 1.1-3.5), and discharge other than home (1.5, 1.1-2.0). Risk factors for those with claudication were dependent functional status (3.5, 1.4-8.7), smoking (1.6, 1.02-2.5), diabetes (1.5, 1.01-2.3), preoperative dialysis (3.6, 1.6-8.3), procedure time exceeding 120 minutes (1.8, 1.1-2.7), in-hospital bleeding (2.9, 1.2-7.4), and in-hospital unplanned return to the operating room (3.4, 1.2-9.4).

Conclusions

Unplanned readmission after endovascular treatment is relatively common, especially in patients with CLTI, and is associated with substantially increased mortality. Awareness of these risk factors will help providers identify patients at high-risk who may benefit from early surveillance and prophylactic measures focused on decreasing postoperative complications may reduce the rate of readmission.

INTRODUCTION

Unplanned readmissions following surgical intervention are associated with poor outcomes and additional healthcare costs.[1] In 2004, it was estimated that the costs of potentially avoidable re-hospitalizations were as high as 17.4 billion dollars among Medicare beneficiaries.[2] Consequently, several regional and national initiatives began focusing on this issue.[3,4] The federal government also made this issue a priority with the Hospital Readmission Reduction Program, introduced in 2012 as a part of the Affordable Care Act, which was initiated to impose financial penalties against hospitals with excessive readmissions within 30 days of discharge for Medicare beneficiaries. An algorithm was developed to omit planned readmissions from the penalty calculation; however, hospitals are accountable for all-cause unplanned readmissions, including those not related to the initial admission. While the readmission rates nationwide remained stable between 2007-2011, after initiation of this policy, rates declined slightly by 0.5%.[5] Within this act, conditions known to have a particularly high risk of readmission (myocardial infarction, heart failure, and pneumonia) were identified as target areas, with vascular procedures as a likely target in the near future. Subsequently, the readmission rate continued to decline in 2015 for both targeted and non-targeted conditions, with 17.8% and 13.1% of all Medicare beneficiaries readmitted within 30 days, respectively.[6]

Readmission following vascular surgery is higher than other major operations, with prior reports citing a range of 18% to 24%, compared to 10% for general, bariatric, and colorectal surgery.[1,2] Among vascular surgery patients, those undergoing lower extremity open or endovascular procedures have amongst the highest risk for readmission with a reported rate of 23%, third only to congestive heart failure and psychoses. Previous studies have identified several risk factors following infrainguinal bypass surgery, including: age, diabetes, renal insufficiency, critical limb ischemia, return to the operating room during the index admission, and longer hospital stay.[7-12] Despite endovascular procedures now being the most commonly used method for lower extremity revascularization, predictors of readmission after infrainguinal endovascular intervention are limited.[13,14] While Davenport et al. analyzed readmission data for both open and endovascular interventions, no predictors specific to endovascular procedures were identified.[15] In addition, Vogel et al. only evaluated outcomes in a limited set of patients who underwent tibioperoneal angioplasty for chronic limb-threatening ischemia in the US Medicare population.[16]

Therefore, the purpose of this study was to assess the incidence of 30-day unplanned readmission following infrainguinal endovascular intervention and to identify preoperative and in-hospital risk factors using a large national representative clinical registry.

METHODS

Data source

Patients were identified using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) Targeted Vascular module. NSQIP is a prospective clinical registry of surgical perioperative outcomes collected nationwide for the purpose of quality assessment and improvement. The registry consists of patient demographic, operative, and postoperative variables up to 30 days after surgery. Additionally, the Targeted Vascular module contains detailed anatomic and intraoperative characteristics, as well as procedure-related outcomes from 83 participating sites. According to NSQIP protocol, trained clinical nurses first identify surgical procedures by reviewing operative case logs then collect data and categorize these procedures using Current Procedural Terminology (CPT) codes. While not all cases are included, a systematic sampling system was developed by NSQIP to select cases and prevent bias in selection. Data collection and quality control have been validated by annual audits and previous reports.[17-19] In 2011, NSQIP began collecting 30-day readmission events; however, the time to readmission, indication, and whether the readmission was related to the principal procedure only became available in 2012.
Therefore, the data were evaluated for 2012-2014 only. Readmission data captured by NSQIP have been previously validated.[4] More details on this registry can be found at www.acsnsqip.org. The Beth Israel Deaconess Medical Center Institutional Review Board approved this study with informed consent waived due to the de-identified nature of NSQIP.

Patients

Patients undergoing an infrainguinal endovascular intervention as a principal procedure were included (CPT: 37224-37233). Extra procedural detail regarding treatment approach was not captured by NSQIP, which precluded us from determining whether the endovascular intervention was a percutaneous or an open procedure. Those not at risk for readmission due to death during the index admission (N=75; 1.7%) or patients with a hospital stay longer than 30 days (N=51; 1.1%) were excluded from the analysis. We stratified patients by symptom status, either intermittent claudication or chronic limb-threatening ischemia (CLTI). Patients with and without unplanned readmission were compared on baseline characteristics, intraoperative details, and postoperative outcomes.

Pre- and intraoperative characteristics

Baseline characteristics consisted of demographics, comorbidities, and pre-procedural medication. Age was considered as a categorical and a continuous variable; however, all patients 90 years of age or older are recorded as 90+ by NSQIP to prevent individual patient identification. Smoking included current tobacco use or within the last year prior to intervention. Preoperative documentation of an open wound or infection was captured, although the location could not be determined with NSQIP data. Dependent functional status was defined as partial or total assistance for activities of daily living (ADL) captured within

30 days prior to the procedure. Intraoperative details captured were type of procedure, the American Society of Anesthesiologist (ASA) class, procedure time, and whether the procedure was elective. Concurrent procedures were identified with corresponding CPT codes (Supplementary table I); however, we were unable to distinguish whether the concomitant procedure was ipsilateral or contralateral based on these billing codes.

Readmissions

The primary outcome was any unplanned readmission within 30 days of the principal endovascular procedure to any hospital, which will be referred to as unplanned readmission in this manuscript. Unplanned readmission was captured by a NSQIP variable and excluded elective or planned readmissions. In addition, unplanned readmissions were further categorized within NSQIP as related or unrelated to the principal procedure and this distinction cannot be made for planned readmissions. Related unplanned readmission indications were determined with a specific list of NSQIP complications or International Classification of Diseases (ICD-9) codes (Supplementary table II). Limb-related readmissions were defined as all readmissions related to the ipsilateral index limb or endovascular procedure. An infection or wound complication refers to a composite variable using ICD-9 codes, which subsequently does not allow us to differentiate between pre-existing wounds or postoperative complications. We defined recurrent symptoms of peripheral vascular disease as worsening of peripheral vascular symptoms likely related to atherosclerotic disease, stenosis, occlusion, or failure of treatment.

Outcomes

Other postoperative outcomes included mortality after index admission and 30-day adverse events. Both 30-day and in-hospital (during the index admission) complications were captured, provided that a time-to-event variable was documented. Major adverse limb event (MALE) refers to a composite variable containing major amputation (above or below knee) and/or major reintervention (new or revision bypass graft, thrombectomy or thrombolysis, or major surgical revision such as jump or interposition graft). Surgical site infection or complication included any surgical site infection or wound dehiscence. Bleeding was defined as any transfusion or secondary procedure with the indication of bleeding. A respiratory complication was considered present when one of the following was documented: pneumonia, unplanned reintubation, or ventilator requirement >48 hours. Any complication included myocardial infarction, stroke, bleeding, surgical site infection or complication, renal deterioration (creatinine >2mg/dL and/or need for dialysis), respiratory complications, sepsis, septic shock, untreated loss of patency, and unplanned return to the operating room. Discharge other than home was defined as any discharge to a skilled or unskilled nursing facility in those patients who were originally admitted from home. These variable definitions were determined by NSQIP prior to data collection and thus were not modifiable.

Statistical analysis

Differences between patients with and without unplanned readmission were determined using the Pearson's χ^2 and Fisher's exact test for categorical variables, while continuous variables were analyzed by Student's t-test and Mann Whitney U test, where appropriate. Multivariable logistic regression was performed to establish independent associations with 30-day unplanned readmission. For adjusted analysis, models were constructed using purposeful selection of covariates, incorporating backward elimination after univariate testing (P-value < .10).[20] Separate regression models were constructed for patients with CLTI and claudication and the cut-off value for procedure time and length of stay was the 75th percentile. Statistical significance was defined as P-value < .05 (two-sided test). Analyses were conducted with SPSS Statistics 23 (IBM Corp, Armonk, NY) .

RESULTS

A total of 4,449 patients were included in the study, of which 63% (N=2,802) had CLTI (66% of these had tissue loss) and 37% (N=1,647) had claudication. Among patients with CLTI, the 30-day unplanned readmission rate was 16% (N=447), and of those patients 5.1% (N=23) were readmitted more than once. The unplanned readmission rate in patients with claudication was 6.5% (N=107), and of those patients 2.8% (N=3) had more than one readmission. To put this into perspective, planned readmissions within 30 days occurred in 2.2% (N=62) for CLTI patients and in 1.5% (N=25) in those with claudication.

Baseline characteristics

Table I summarizes patients' baseline characteristics. Compared to CLTI patients without an unplanned readmission, those with an unplanned readmission were less likely to be white (53% vs. 60%, P = .04), more often functionally dependent (19% vs. 15%, P = .02), and more frequently had tissue loss (71% vs. 65%, P = .02). Patients who were readmitted more often had diabetes (70% vs. 61%, P < .001), congestive heart failure (7.4% vs. 3.9%, P = .001), renal insufficiency (49% vs. 31%, P < .001), dialysis (24% vs. 12%, P < .001), and a preoperative open wound or infection (60% vs. 51%, P < .001). Finally, among patients with CLTI, preoperative use of statin (73% vs. 67%, P = .02) and beta blocker medications (71% vs. 65%, P = .01) were more frequent in those readmitted.

In claudication patients, unplanned readmission was associated with dependent functional status (6.6% vs. 1.6%, P < .001), diabetes (51% vs. 40%, P = .02), dialysis (8.4% vs. 2.3%, P < .001), preoperative open wound or infection (16% vs. 3.8%, P < .001), and preoperative use of beta blocker medications (66% vs. 57%, P = .047).

Table I. Baseline characteristics and comorbidities among chronic limb-threatening ischemia (CLTI) and claudication patients with and without an unplanned readmission following endovascular intervention

	CLTI					Claudication				
	Unplanned Readmission (N=447)		No readmission (N=2355)		P-value	Unplanned Readmission (N=107)		No readmission (N=1540)		P-value
	N	%	N	%		N	%	N	%	
Male gender	254	(57)	1330	(57)	.89	63	(59)	957	(62)	.50
Age - years (mean ± SD)	70.0 (12)		69.9 (12)		.90	67.0 (10)		67.8 (11)		48
Age categories					.83					.47
< 60	93	(21)	514	(22)		22	(21)	342	(22)	
60 to 69	124	(28)	612	(26)		43	(40)	507	(33)	
70 to 79	124	(28)	635	(27)		32	(30)	469	(31)	
80 to 89	84	(19)	487	(21)		9	(8.4)	202	(13)	
> 89	22	(4.9)	107	(4.5)		1	(0.9)	20	(1.3)	
Smoking	109	(24)	641	(27)	.22	48	(45)	564	(37)	.09
BMI > 30	128	(30)	677	(30)	.96	32	(31)	544	(36)	.32
Race					**.04**					.30
White	236	(53)	1409	(60)		69	(65)	1099	(71)	
Black	102	(23)	441	(19)		19	(18)	220	(14)	
Hispanic	61	(14)	258	(11)		10	(9.3)	79	(5.1)	
Other	12	(2.7)	45	(1.9)		1	(0.9)	19	(1.2)	
Unknown/not reported	36	(8.1)	202	(8.6)		8	(7.5)	123	(8)	
Symptom status					**.02**					
CLTI: rest pain	131	(29)	827	(35)						
CLTI: tissue loss	316	(71)	1528	(65)						
Not admitted from home	71	(16)	345	(15)	.50	3	(2.8)	24	(1.6)	.42
Dependent functional status	84	(19)	346	(15)	**.02**	7	(6.6)	25	(1.6)	**<.001**
Hypertension	397	(89)	2010	(85)	.05	94	(88)	1286	(84)	.24
Diabetes	312	(70)	1429	(61)	**<.001**	55	(51)	620	(40)	**.02**
CHF	33	(7.4)	92	(3.9)	**.001**	4	(3.7)	24	(1.6)	.10
Renal insufficiency	215	(49)	701	(31)	**<.001**	24	(25)	246	(17)	.05
Dialysis	106	(24)	282	(12)	**<.001**	9	(8.4)	36	(2.3)	**<.001**
COPD	51	(11)	227	(9.6)	.25	12	(11)	170	(11)	.96
History of revascularization in same segment					.12					.66
No prior revascularization	288	(64)	1407	(60)		58	(54)	879	(57)	
Prior bypass surgery	74	(17)	403	(17)		19	(18)	290	(19)	
Prior endovascular intervention	85	(19)	545	(23)		30	(28)	371	(24)	
Preprocedural medication										
Antiplatelet	365	(82)	1924	(82)	.80	90	(84)	1345	(88)	.25
Statin	323	(73)	1566	(67)	**.02**	76	(71)	1135	(74)	.45
Beta blocker	317	(71)	1517	(65)	**.01**	71	(66)	860	(57)	**.047**
Preoperative open wound or infection	269	(60)	1199	(51)	**<.001**	17	(16)	58	(3.8)	**<.001**

SD: standard deviation, *BMI*: body mass index, *CHF*: congestive heart failure, *COPD*: chronic obstructive pulmonary disease

Procedure details

Compared to CLTI patients without an unplanned readmission, those who were readmitted had a significantly longer procedure time (102 min vs. 92 min, P < .01; Table II), and were less likely to undergo an elective procedure (48% vs. 54%, P = .03). Similarly, ASA class ≥ 4 (29% vs. 21%, P < .001) and tibial angioplasty and/or stenting (34% vs. 26%, P = .001) were more common among readmitted CLTI patients. In the vast majority of cases, vascular surgeons performed the procedure as opposed to other specialties (92% vs. 8%).

Among patients with claudication, those with unplanned readmissions more often underwent tibial angioplasty and/or stenting compared to those without an unplanned readmission (14% vs. 8.4%, P = .046). In addition, readmitted patients had a longer procedure time (102 min vs. 81 min, P < .001), and more frequently had an ASA class ≥ 4 (24% vs. 8.9%, P < .001). Vascular surgeons performed more endovascular procedures than other specialties (85% vs. 15%). Regarding concurrent procedures, readmitted patients with claudication more often underwent an additional thromboendarterectomy compared to those not readmitted (13% vs. 5.4%, P = .001).

Table II. Procedure details among chronic limb-threatening ischemia (CLTI) and claudication patients with and without an unplanned readmission following endovascular intervention

	CLTI					**Claudication**				
	Unplanned Readmission *(N=447)*		**No readmission** *(N=2355)*		**P-value**	**Unplanned Readmission** *(N=107)*		**No readmission** *(N=1540)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Type procedure					**.001**					**.046**
Fem-pop angioplasty/stenting/ atherectomy	295	(66)	1734	(74)		92	(86)	1411	(92)	
Tibial angioplasty/stenting	152	(34)	621	(26)		15	(14)	129	(8.4)	
Elective procedure	215	(48)	1267	(54)	**.03**	79	(75)	1377	(89)	**<.001**
Concurrent procedures										
Open bypass	1	(0.2)	12	(0.5)	.71	2	(1.9)	7	(0.5)	.11
Thromboendarterectomy	28	(6.3)	137	(5.8)	.71	14	(13)	83	(5.4)	**.001**
ASA class ≥ 4	115	(29)	436	(21)	**<.001**	22	(24)	119	(8.9)	**<.001**
Procedure time - (min ± IQR)	102 (66-148)		92 (63-136)		**<.01**	102 (62-161)		81 (58-118)		**<.001**
Fem-pop angioplasty/stenting/ atherectomy	101 (66-154)		93 (62-139)		.06	99 (61-190)		80 (57-119)		**<.001**
Tibial angioplasty/stenting	103 (67-141)		89 (65-129)		**.02**	103 (74-137)		82 (62-117)		.12

ASA: American Society of Anesthesiologists, *min*: minutes, *IQR*: interquartile range

Postoperative outcomes

Postoperative outcomes are detailed in Table III. Mortality after index admission was higher in readmitted patients for both CLTI (3.4% vs. 0.7%, P < .001) and claudication (2.8% vs. 0.1%, P < .01). Time to readmission occurred at a median of 12 days after discharge in patients with CLTI (IQR: 6-19) and 13 days in those with claudication (IQR: 7-18). The 30-day rate of any complication in CLTI patients with an unplanned readmission was higher than in those not readmitted (61% vs. 15%, P < .001). Readmitted patients with claudication also suffered more complications (66% vs. 4.9%, P < .001). In patients with an unplanned readmission, the following 30-day adverse events were more frequently found: MALE, major amputation, major reintervention, surgical site infection or complication, bleeding requiring transfusion or secondary procedure, myocardial infarction, stroke, progressive renal insufficiency and/or dialysis, respiratory complications, sepsis, and unplanned return to the operation room. In-hospital postoperative adverse events associated with unplanned readmission in patients with CLTI and claudication were similar and included bleeding requiring transfusion or secondary procedure (CLTI: 9.2% vs. 6.1%, P = .02; claudication: 8.4% vs. 1.6%, P < .001) and unplanned return to the operating room (CLTI: 9.4% vs. 5.9%, P < .01; claudication: 6.5% vs. 1.2%, P < .001). The median length of hospital stay was 1 day among patients with CLTI and claudication (Figure I), although those readmitted had a longer hospital stay (CLTI: 2 days vs. 1 day, P < .01; claudication: 1 day vs. 0 days, P < .001).

Indications for readmission

Of all unplanned readmissions, approximately half were considered related to the index endovascular procedure in patients with CLTI (49%) and claudication (53%). Indications for readmission varied by symptom status and are listed in Table IV. In CLTI patients, readmissions were limb-related in 72%, which included 42% infection or wound complications, and 14% due to recurrent symptoms of peripheral vascular disease. Readmission indications in patients with claudication were also primarily limb-related (68%), with 8.8% readmitted due to infection or wound complications and 28% of patients readmitted for recurrent symptoms of peripheral vascular disease.

Predictors of unplanned readmission

Multivariable predictors of 30-day unplanned readmission are listed in Table V. Preoperative independent risk factors of any unplanned readmission in patients with CLTI were diabetes (OR: 1.3, 95% CI: 1.01-1.6), congestive heart failure (OR: 1.6, 95% CI: 1.1-2.5), renal insufficiency (OR: 1.7, 95% CI: 1.3-2.2), and preoperative dialysis (OR: 1.4, 95% CI: 1.02-1.9). Intraoperatively, tibial angioplasty and/or stenting (OR: 1.3, 95% CI: 1.04-1.6) was also associated with an increased risk of unplanned readmission. Additional pre-discharge risk factors were in-hospital bleeding (OR: 1.9, 95% CI: 1.04-3.5) and unplanned return to the operating room (OR: 1.9, 95% CI: 1.1-3.5). Finally, discharge other than home was associated with unplanned readmission in CLTI patients (OR: 1.5, 95% CI: 1.1-2.0).

Table III. Postoperative outcomes among chronic limb-threatening ischemia (CLTI) and claudication patients with and without an unplanned readmission following endovascular intervention

	CLTI					Claudication				
	Unplanned Readmission *(N=447)*		**No readmission** *(N=2355)*		**P-value**	**Unplanned Readmission** *(N=107)*		**No readmission** *(N=1540)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Mortality after index admission	15	(3.4)	17	(0.7)	**<.001**	3	(2.8)	2	(0.1)	**<.01**
Length of stay - d (median ± IQR))	2 (1-5)		1 (1-4)		**<.01**	1 (0-2)		0 (0-1)		**<.001**
Discharge to home	334	(83)	1952	(88)	**.001**	99	(95)	1510	(98)	**.02**
30-day MALE	118	(26)	130	(5.5)	**<.001**	34	(32)	12	(0.8)	**<.001**
Major amputation	68	(15)	75	(3.2)	**<.001**	5	(4.7)	4	(0.3)	**<.001**
Major reintervention	57	(13)	69	(2.9)	**<.001**	30	(28)	10	(0.6)	**<.001**
Surgical site infection or complication	50	(11)	41	(1.7)	**<.001**	11	(10)	14	(0.9)	**<.001**
In-hospital	5	(1.1)	11	(0.5)	.09	0	(0)	3	(0.2)	**>.99**
Myocardial infarction	13	(2.9)	22	(0.9)	**.001**	3	(2.8)	5	(0.3)	**.01**
In-hospital	1	(0.2)	20	(0.8)	.23	0	(0)	5	(0.3)	>.99
Stroke	1	(0.2)	4	(0.2)	.58	3	(2.8)	1	(0.1)	**.001**
In-hospital	0	(0)	4	(0.2)	>.99	0	(0)	1	(0.1)	>.99
Bleeding (requiring transfusion or secondary procedure)	79	(18)	160	(6.8)	**<.001**	26	(24)	31	(2)	**<.001**
In-hospital	41	(9.2)	143	(6.1)	**.02**	9	(8.4)	24	(1.6)	**<.001**
Creatinine >2 mg/dL	12	(2.7)	11	(0.5)	**<.001**	1	(0.9)	1	(0.1)	.13
In-hospital	2	(0.4)	11	(0.5)	>.99	1	(0.9)	1	(0.1)	.13
New dialysis dependence	5	(1.1)	3	(0.1)	**<.01**	1	(0.9)	1	(0.1)	.13
In-hospital	1	(0.2)	3	(0.1)	.50	1	(0.9)	1	(0.1)	.13
Respiratory complications	24	(5.4)	23	(1)	**<.001**	3	(2.8)	1	(0.1)	**.001**
In-hospital	3	(0.7)	21	(0.9)	>.99	1	(0.9)	1	(0.1)	.13
Sepsis	20	(4.5)	10	(0.4)	**<.001**	2	(1.9)	0	(0)	**<.01**
In-hospital	0	(0)	9	(0.4)	.37	1	(0.9)	0	(0)	.07
Septic shock	8	(1.8)	2	(0.1)	**<.001**	3	(2.8)	0	(0)	**<.001**
In-hospital	1	(0.2)	1	(0)	.29	1	(0.9)	0	(0)	.07
Untreated loss of patency	13	(2.9)	35	(1.5)	**.03**	2	(1.9)	10	(0.6)	.18
In-hospital	1	(0.2)	14	(0.6)	.49	0	(0)	1	(0.1)	>.99
Unplanned return to the operating room	198	(44)	174	(7.4)	**<.001**	56	(52)	29	(1.9)	**<.001**
In-hospital	42	(9.4)	139	(5.9)	**<.01**	7	(6.5)	19	(1.2)	**<.001**
Any complication	274	(61)	362	(15)	**<.001**	71	(66)	76	(4.9)	**<.001**
In-hospital	70	(16)	271	(12)	**.01**	13	(12)	42	(2.7)	**<.001**

d: days, *IQR*: interquartile range, *MALE:* major adverse limb event

Among patients with claudication, predictors of any unplanned readmission included dependent functional status (OR: 3.5, 95% CI: 1.4-8.7), smoking (OR: 1.6, 95% CI: 1.02-2.5), diabetes (OR: 1.5, 95% CI: 1.01-2.3), preoperative dialysis (OR: 3.6, 95% CI: 1.6-8.3), and procedure time exceeding 120 minutes (OR: 1.8, 95% CI: 1.1-2.7). Similar to patients with CLTI, unplanned readmission in those with claudication was also independently associated with pre-discharge complications, such as in-hospital bleeding (OR: 2.9, 95% CI: 1.2-7.4) and in-hospital unplanned return to the operating room (OR: 3.4, 95% CI: 1.2-9.4).

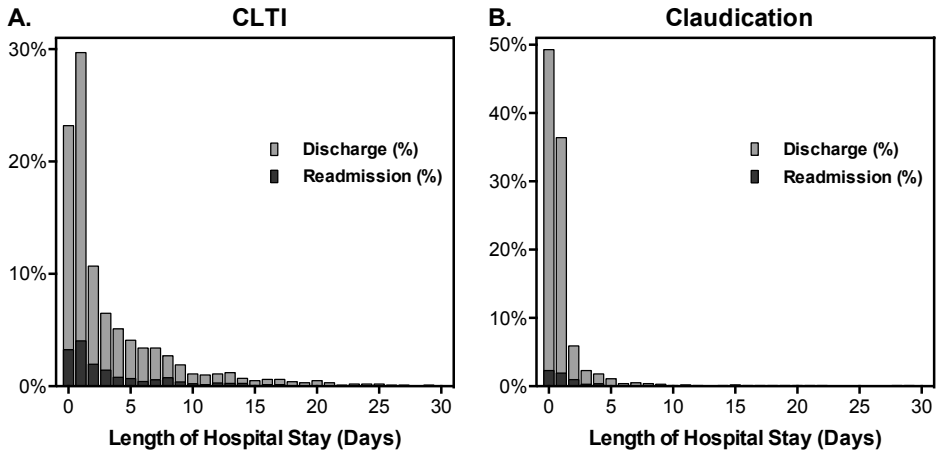

Figure 1. Proportions of discharge and readmission at different lengths of hospital stay after endovascular intervention for patients with **A**. chronic limb-threatening ischemia (CLTI) and **B**. claudication

Table IV. Indications of 30-day unplanned related readmissions among patients with chronic limb-threatening ischemia (CLTI) and claudication following endovascular intervention

	CLTI		**Claudication**	
	(N=220)		*(N=57)*	
	N	*%*	*N*	*%*
Limb-related readmissions	158	(72)	39	(68)
Infection or wound complications	92	(42)	5	(8.8)
Recurrent symptoms of peripheral vascular disease	30	(14)	16	(28)
Other vascular complications	36	(16)	18	(32)
Sepsis	12	(5.5)	3	(5.3)
Other readmissions	45	(21)	12	(21)
Unknown indication	5	(2.3)	3	(5.3)

Table V A. Independent predictors of 30-day unplanned readmission following endovascular intervention in patients with chronic limb-threatening ischemia (CLTI)

		CLTI	
Variable	**OR**	**95% CI**	**P-value**
Diabetes	1.3	1.01-1.6	**.04**
Congestive heart failure	1.6	1.1-2.5	**.03**
Renal insufficiency	1.7	1.3-2.2	**<.001**
Preoperative dialysis	1.4	1.02-1.9	**.04**
Tibial angioplasty/stenting	1.3	1.04-1.6	**.02**
In-hospital bleeding	1.9	1.04-3.5	**.04**
In-hospital unplanned return to the operation room	1.9	1.1-3.5	**.03**
Discharge other than home	1.5	1.1-2.0	**<.01**

Adjusted for: age, gender, symptom status, race, history of ipsilateral revascularization, procedure time exceeding 140 min (75th percentile), length of stay longer than 4 days (75th percentile), in-hospital surgical site infection or complication

Table V B. Independent predictors of 30-day unplanned readmission following endovascular intervention in patients with claudication

		Claudication	
Variable	**OR**	**95% CI**	**P-value**
Dependent functional status	3.5	1.4-8.7	**<.01**
Smoking	1.6	1.02-2.5	**.04**
Diabetes	1.5	1.01-2.3	**.048**
Preoperative dialysis	3.6	1.6-8.3	**<.01**
Procedure time exceeding 120 min (75th percentile)	1.8	1.1-2.7	**.01**
In-hospital bleeding	2.9	1.2-7.4	**.02**
In-hospital unplanned return to the operation room	3.4	1.2-9.4	**.02**

Adjusted for: age, gender

DISCUSSION

This study demonstrates that 30-day unplanned readmission after infrainguinal endovascular intervention is relatively common for CLTI (1 in 6 patients) and claudication (1 in 15 patients). Also, mortality and morbidity after index admission were significantly worse in those readmitted. Adjusted analysis demonstrated that diabetes, congestive heart failure, renal insufficiency, preoperative dialysis, and tibial angioplasty and/or stenting were independent predictors of unplanned readmission in CLTI patients. Other risk factors included postoperative adverse events, such as in-hospital bleeding, in-hospital unplanned return to the operating room, and discharge other than home. Among patients with claudication,

risk factors for unplanned readmission were dependent functional status, smoking, diabetes, preoperative dialysis, and procedure time exceeding 120 minutes, as well as in-hospital factors, such as bleeding and unplanned return to the operating room.

Several other studies have evaluated readmission rates of endovascular procedures, although few of these stratified by symptom status and whether a readmission was planned or unplanned. An analysis between 2008-2010 of the Health Facts database, which records longitudinal patient data from over 480 contributing institutions throughout the U.S., found a readmission rate of 19% in 251 patients with CLTI and 11% in 221 claudication patients undergoing endovascular intervention, whereas a single-institution study of 137 endovascular interventions, showed that 12% of CLTI and 2.2% of claudication patients were readmitted.[21,22] Our rates of 16% in patients with CLTI and 6.5% in those with claudication fall well within these reported ranges. The variation may be partly attributable to differences in follow-up time, namely 30 days from discharge (both previous studies) rather than from the procedure, which was assessed in the current study. Our rates may be higher than those reported from Jackson et al.[22] as NSQIP captures readmissions even at different institutions, therefore providing a more complete representation of all readmissions. While the 30-day time period is limited, we believe that it still represents a critical time period, especially in light of the short time between an endovascular procedure and typical discharge.

When comparing our results to studies conducted among patients undergoing infrainguinal bypass surgery, we found similar predictors of readmission.[7-12] Only one other study, by Vogel et al., identified predictors of readmission following tibioperoneal angioplasty using the US Medicare database and showed that gangrene, chronic heart failure, chronic obstructive pulmonary disease, and renal failure were independent risk factors of 30-day unplanned readmission in CLTI patients.[16] The association between renal failure and higher risk of readmission has been demonstrated previously,[2,9,12,15,21] and the present study further supports that end-stage renal disease correlates with worse outcomes, in particular a 1.4- and 3.6-fold increased risk of readmission in patients with CLTI and claudication, respectively. While the study by Vogel et al. included over 13,000 patients, Medicare uses administrative discharge data for billing purposes, which limits the ability to appropriately capture comorbidities, functional status, and operative details. In contrast, NSQIP is not an administrative database but rather a clinical registry that uses clinical reviewers to evaluate operative and progress notes, and also gathers complete 30-day follow-up data through outpatient chart review and telephone follow-up.

There are important clinical implications to this study. Many preoperative risk factors are not directly modifiable; however, this study merely reinforces the importance of addressing chronic issues and optimizing co-existing conditions. Furthermore, attention should be focused on procedure-related and in-hospital complications as these are more modifiable and may provide opportunities to further lower readmission rates. Previous studies reported similar postoperative complications, such as bleeding and unplanned return to the operating room as risk factors,[12,15] which may be attributed to anatomy- or

procedure-specific difficulties. However, given their strong association with readmission, care should be taken to minimize blood loss and in the occurrence of complications, close monitoring and evaluation prior to discharge may be appropriate, such as duplex evaluation for pseudoaneurysm in the event of a groin hematoma. Consistent with findings of others,[8,15,23-25] our data showed an association between readmission and discharge destination. This is likely related to more advanced comorbidities and worsening functional status, which may not be modifiable. However, advancements in communication between physicians at the hospital and healthcare professionals at skilled nursing facilities both at time of discharge and after may help to improve transition of care and patient outcomes.[26]

Caution is warranted in the use of invasive interventions in patients with claudication, especially tibial revascularization and procedures in those with a poor functional health status, given that these were associated with readmission. In addition, risk reduction guidelines or programs, such as smoking cessation, are associated with reduced postoperative complications and limb events,[27,28] and likely improve outcomes of patients with claudication. Some have suggested withholding surgical treatment until the patient has stopped smoking, which is adopted by the Society of Vascular Surgery (SVS) Lower Extremity Guidelines Committee that recommends multidisciplinary smoking cessation efforts in those with claudication, repeatedly before interventions.[29]

In patients with CLTI, the majority of hospital readmissions identified in this study were related to infection and wound complication, with 42% of all readmissions. Other studies have shown similar readmission rates due to infectious complications after infrainguinal revascularization, ranging from 38 to 55%.[11,12,15,25] As wound complications are likely related to a number of patient factors, including diabetes, we believe this may account for some of the increased risk of wound complications. Additionally, open concurrent procedures may attribute to the increased risk and are likely to represent challenging anatomy or access site complications due to artery calcification, small vessel diameter, or previous interventions. Involvement of case managers for effective patient education and discharge instructions about potential complications may help further reduce postoperative readmissions.

Time to readmission is another consideration in lowering readmission rates. Merkow et al. reported that readmissions after surgical procedures appeared evenly dispersed over time with no particular peak following discharge.[25] In our analysis, patients with CLTI and claudication were readmitted at a median of 12 and 13 days, respectively. As addressed previously, early medical follow-up may be strategically timed.[2] Capturing adverse events during early surveillance may allow for treatment in the outpatient setting, for example by nurse-conducted telephone calls 1-week post discharge or improved utilization of skilled nursing facilities. Moreover, identifying high-risk patients can help to prioritize patients who should be seen in clinic sooner after discharge.

While we found higher readmissions rates in those patients with a longer hospital stay, no association was demonstrated in the adjusted analysis. Some may argue that keeping patients admitted for a few additional days may avert potential readmissions, although

whether this reduces post discharge complications remains to be seen. Given that NSQIP follow-up ends at 30 days post intervention, a longer length of stay shortens post discharge follow-up and necessarily reduces the patient's time at risk of readmission. Therefore, this study was unable to provide an accurate estimate in regard to the incidence of readmission when considering hospital stay duration, albeit short after endovascular intervention.

This study has several limitations that should be addressed. First, as NSQIP collects data through a clinical registry, it is subjected to data misclassification and limited by variable definitions. Second, confounding by indication may occur, since patients undergoing angioplasty were potentially unsuitable for open bypass surgery, despite adjustment for all reported baseline characteristics in the multivariable analysis. In addition, this study was unable to assess the lesion severity, extent of PAD, and the location of the puncture site or incision (e.g. groin, arm, lower leg), which will likely impact outcomes. Nonetheless, we believe that the Targeted Vascular module of NSQIP is a nationally representative and robust clinical registry that allows for reliable risk assessment in the 30-day postoperative period. As this study can only assess 30-day outcomes, further study is needed to identify the full implications of unplanned readmissions on a patient and national level. The SVS Vascular Quality Initiative registry provides more detailed variables, including closure device and ultrasound use, that may further explain associations found in this study.

CONCLUSIONS

This study demonstrates that unplanned readmission following endovascular intervention is relatively common and associated with increased morbidity and mortality. In both patients with CLTI and claudication, risk factors for unplanned readmission include patient characteristics and procedure-related factors, as well as occurrence of in-hospital complications such as bleeding and unplanned return to the operating room. Many readmissions may not be avoidable, particularly in the CLTI population, due to the extent of their comorbidities and socioeconomic status. However, this should not dissuade us from making efforts to optimize co-existing conditions, involve case managers to improve post discharge care and consider nurse-conducted phone calls to assess for potential complications that may be managed in an outpatient setting if identified early.

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SUPPLEMENTARY

Supplementary table I. Concurrent open procedures with corresponding Current Procedural Terminology (CPT) codes

CPT codes	Definition CPT codes
Open bypass	
35556	Bypass graft, with vein; femoral-popliteal
35565	Bypass graft, with vein; iliofemoral
35566	Bypass graft, with vein; femoral-anterior tibial, posterior tibial, peroneal artery or other distal vessels
35570	Bypass graft, with vein; tibial-tibial, peroneal-tibial, or tibial/peroneal trunk-tibial
35571	Bypass graft, with vein; popliteal-tibial, -peroneal artery or other distal vessels
35583	In-situ vein bypass; femoral-popliteal
35585	In-situ vein bypass; femoral-anterior tibial, posterior tibial, or peroneal artery
35587	In-situ vein bypass; popliteal-tibial, peroneal
35651	Bypass graft, with other than vein; aortofemoral-popliteal
35656	Bypass graft, with other than vein; femoral-popliteal
35661	Bypass graft, with other than vein; femoral-femoral
35665	Bypass graft, with other than vein; iliofemoral
35666	Bypass graft, with other than vein; femoral-anterior tibial, posterior tibial, or peroneal artery
35671	Bypass graft, with other than vein; popliteal-tibial or -peroneal artery
Thromboendarterectomy	
35302	Thromboendarterectomy, including patch graft, if performed; superficial femoral artery
35303	Thromboendarterectomy, including patch graft, if performed; popliteal artery
35304	Thromboendarterectomy, including patch graft, if performed; tibioperoneal trunk artery
35305	Thromboendarterectomy, including patch graft, if performed; tibial or peroneal artery, initial vessel
35306	Thromboendarterectomy, including patch graft, if performed; each additional tibial or peroneal artery (List separately in addition to code for primary procedure)
35351	Thromboendarterectomy, with or without patch graft; iliac
35355	Thromboendarterectomy, with or without patch graft; iliofemoral
35371	Thromboendarterectomy, with or without patch graft; common femoral
35372	Thromboendarterectomy, with or without patch graft; deep (profunda) femoral
35381	Thromboendarterectomy, with or without patch graft; femoral and/or popliteal, and/or tibioperoneal

Supplementary table II. Indications of related unplanned readmissions based on NSQIP complication variables and International Classification of Diseases (ICD)-9 codes

Variables	NSQIP complication variables	ICD-9 code	Definition ICD-9 code
Limb-related readmission			
	Infection or wound complication		
	Superficial Incisional SSI	440.23	Atherosclerosis of native arteries of the extremities with ulceration
	Deep Incisional SSI	440.24	Atherosclerosis of native arteries of the extremities with gangrene
	Organ/Space SSI	681.10	Unspecified cellulitis and abscess toe
	Wound Disruption	682.6	Cellulitis and abscess of leg except foot
		682.7	Cellulitis and abscess of leg except toes
		682.9	Cellulitis and abscess of unspecified sites
		686.9	Unspecified local infection of skin and subcutaneous tissue
		707.15	Ulcer of other part of foot
		730.07	Acute osteomyelitis involving ankle and foot
		785.4	Gangrene
		997.62	Infection (chronic) of amputation stump
		998.30	Disruption of wound
		998.59	Other postoperative infection
		998.83	Non-healing surgical wound
		L03.115	Cellulitis of right lower limb
	Recurrent symptoms of peripheral vascular disease		
		440.20	Atherosclerosis of native arteries of the extremities unspecified
		440.21	Atherosclerosis of native arteries of the extremities with intermittent claudication
		440.22	Atherosclerosis of native arteries of the extremities with rest pain
		440.29	Other atherosclerosis of native arteries of the extremities
		440.30	Atherosclerosis of unspecified bypass graft
		440.31	Atherosclerosis of autologous vein bypass graft
		440.4	Chronic total occlusion of artery of the extremities
		443.9	Peripheral vascular disease unspecified
		444.22	Arterial embolism and thrombosis of lower extremity
		459.9	Peripheral occlusion combined with diabetes
		729.5	Pain in limb
		729.81	Swelling of limb

Supplementary table II. *Continued*

Variables	NSQIP complication variables	ICD-9 code	Definition ICD-9 code
Limb-related readmission			
Other vascular complications			
	Bleeding Requiring Transfusion	338.18	Other acute postoperative pain
	DVT Requiring Therapy	442.30	Aneurysm of artery of lower extremity
		453.41	Acute venous embolism and thrombosis of deep vessel of proximal lower extremity
		996.1	Mechanical complication of other vascular device, implant, and graft
		996.59	Mechanical complication of other implant and internal device not elsewhere classified
		996.74	Other complications due to other vascular device, implant, and graft
		997.2	Peripheral vascular complications not elsewhere classified
		997.69	Other late amputation stump complication
		998.11	Hemorrhage complicating a procedure
		998.12	Hematoma complicating a procedure
		998.13	Seroma complicating a procedure
		E878.5	Amputation of limb(s) causing abnormal patient reaction or later complication without misadventure at time of operation
		V49.75	Below knee amputation status
Sepsis			
	Sepsis	038.9	Unspecified septicemia (sepsis)
	Septic Shock		
Other readmission			
	Pneumonia	250.60	Diabetes with neurological manifestations, type II or unspecified type, not stated as uncontrolled
	Pulmonary Embolism	250.70	Diabetes with peripheral circulatory disorders, type II or unspecified type, not stated as uncontrolled
	On ventilator > 48 hours	250.80	Diabetes with other specified manifestations, type II or unspecified type, not stated as uncontrolled
	Myocardial Infarction	285.9	Anemia, unspecified
	Cardiac Arrest Requiring CPR	427.31	Atrial fibrillation
	CVA	428.0	Congestive heart failure
	Acute Renal Failure	428.21	Acute systolic heart failure
	Progressive Renal Insufficiency	428.33	Acute on chronic diastolic heart failure
	Urinary Tract Infection	442.90	Other aneurysm of unspecified site
		444.00	Other arterial embolism and thrombosis of abdominal aorta

Supplementary table II. *Continued*

Variables	NSQIP complication variables	ICD-9 code	Definition ICD-9 code
Other readmission			
		458.29	Other iatrogenic hypotension
		459.0	Hemorrhage unspecified
		491.21	Obstructive chronic bronchitis with (acute) exacerbation
		507.0	Pneumonitis due to inhalation of food or vomitus
		518.81	Acute respiratory failure
		531.40	Chronic or unspecified gastric ulcer with hemorrhage without obstruction
		557.0	Acute vascular insufficiency of intestine
		569.85	Angiodysplasia of intestine with hemorrhage
		578.00	Hematemesis
		578.9	Hemorrhage of gastrointestinal tract, unspecified
		730.0	Acute osteomyelitis site unspecified
		780.20	Syncope and collapse
		780.60	Fever, unspecified
		786.05	Shortness of breath
		786.09	Respiratory abnormality other
		786.5	Chest pain, unspecified
		878.50	Open wound of vulva complicated
		790.01	Precipitous drop in hematocrit
		965.09	Poisoning by other opiates and related narcotics

CHAPTER 8

PERIOPERATIVE OUTCOMES OF INFRAINGUINAL BYPASS SURGERY IN PATIENTS WITH AND WITHOUT PRIOR REVASCULARIZATION

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ABSTRACT

Objective

Although an increasing number of patients with peripheral arterial disease undergo multiple revascularization procedures, the effect of prior interventions on outcomes remains unclear. The purpose of this study was to evaluate perioperative outcomes of bypass surgery in patients with and those without prior ipsilateral treatment.

Methods

Patients undergoing non-emergent infrainguinal bypass between 2011 and 2014 were identified in the NSQIP-Targeted Vascular module. After stratification by symptom status (chronic limb-threatening ischemia [CLTI] and claudication), patients undergoing primary bypass were compared to those undergoing secondary bypass. Within the secondary bypass group, further analysis compared prior bypass to prior endovascular intervention. Multivariable logistic regression analysis was used to establish the independent association between prior ipsilateral procedure and perioperative outcomes.

Results

A total of 7,302 patients were identified, of which 4,540 (62%) underwent primary bypass (68% for CLTI), 1,536 (21%) underwent secondary bypass after a previous bypass (75% for CLTI), and 1,226 (17%) underwent secondary bypass after a previous endovascular intervention (72% for CLTI). Prior revascularization on the same ipsilateral arteries was associated with increased 30-day major adverse limb event in patients with CLTI (9.8% vs. 7.4%; OR: 1.4, 95% CI: 1.1-1.7) and claudication (5.2% vs. 2.5%; 2.1, 1.3-3.5). Similarly, secondary bypass was an independent risk factor for 30-day major reintervention (CLTI: 1.4, 1.1-1.8; claudication: 2.1, 1.3-3.5), bleeding (CLTI: 1.4, 1.2-1.6; claudication: 1.7, 1.3-2.4), and unplanned reoperation (CLTI: 1.2, 1.0-1.4; claudication: 1.6, 1.1-2.1), whereas major amputation was increased in CLTI patients only (1.3, 1.0-1.8). Perioperative mortality was not significantly different in patients undergoing secondary compared to primary bypass (CLTI: 1.7% vs. 2.2%, P = .22; claudication: 0.4% vs. 0.6%, P = .76). Among secondary bypass patients with CLTI, those with prior bypass had higher 30-day reintervention rates (7.8% vs. 4.9%; OR: 1.5, 95% CI: 1.0-2.2), but fewer wound infections (7.3% vs. 12%; 0.6, 0.4-0.8) compared to patients with prior endovascular intervention.

Conclusions

Prior revascularization, in both patients with CLTI and claudication, is associated with worse perioperative outcomes compared to primary bypass. Furthermore, prior endovascular intervention is associated with increased wound infections, whereas those with prior bypass had higher reintervention rates. The increasing prevalence of patients undergoing multiple interventions stresses the importance of patient selection for initial treatment and should be factored into subsequent revascularization options in an effort to decrease adverse events.

INTRODUCTION

Peripheral arterial disease (PAD) affects 12-20% of people in the United States older than 65 years and is associated with substantial morbidity and mortality.[1] As the proportion of elderly patients continues to increase, as well as the utilization of endovascular procedures, rates of reintervention for PAD have been steadily rising.[2,3] Among patients undergoing lower extremity bypass surgery in the current era, it is estimated that 22-25% underwent prior ipsilateral endovascular interventions and 13-19% had prior ipsilateral open bypass.[4-6] Despite increased rates of reintervention, the impact of subsequent revascularization procedures has only recently been studied.

Long-term outcomes comparing primary and secondary bypass have been reported with conflicting results. Several studies demonstrated worse outcomes in those patients undergoing secondary bypass, yet others found equivocal long-term outcomes in patients with prior endovascular interventions.[6-12] Interestingly, despite data on the long-term impact of secondary bypass, differences in perioperative outcomes remain unclear. Previous studies suggest that prior unsuccessful treatment is not associated with worse perioperative performance of bypass surgery.[9-12] However, a study of 3,504 patients undergoing bypass surgery, of which 33% were secondary bypass, found prior revascularization to be a risk factor for in-hospital return to the operating room and graft occlusion at discharge.[6] The body of literature on this topic is still limited, most recent studies included only single-institution data with small sample sizes, and were unable to adjust for prior procedure type.

Therefore, the purpose of this study was to assess perioperative outcomes in patients undergoing bypass surgery following prior ipsilateral bypass surgery or endovascular intervention using a large national representative clinical registry.

METHODS

Data source

Data were obtained from the prospectively collected Targeted Vascular module of the American College of Surgeons National Surgical Quality Improvement Program (NSQIP). NSQIP is a national, multi-institutional, quality improvement initiative of academic and community-based centers that provides 30-day outcomes in an effort to improve overall patient care. Standardized definitions capture demographics, comorbidities, intraoperative variables, and 30-day postoperative outcomes in a randomly selected subset of patients at each participating institution. The Targeted Vascular module includes additional disease and procedure-specific characteristics, as well as procedure-related outcomes chosen by vascular surgeons. Trained clinical reviewers identify potential procedures by reviewing operative case logs then collect data and categorize procedures using Current Procedural Terminology (CPT) codes at both the targeted and non-targeted NSQIP. To ensure data quality, NSQIP data collection is validated by rigorous audits as well as comprehensive

studies.[13-15] Further details on NSQIP and the Vascular Targeted module are available on https://www.facs.org/quality-programs/acs-nsqip. This study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board, and informed consent was waived due to the de-identified nature of this registry.

Patients

All patients undergoing a non-emergent infrainguinal bypass between 2011 and 2014 were included. Patients were stratified by symptom status: intermittent claudication vs. chronic limb-threating ischemia (CLTI). Those without documented symptom status and asymptomatic patients were excluded (n=313; 4.1%). Secondary bypass was defined as a new bypass with a prior endovascular intervention or bypass treating the same ipsilateral arteries as the current procedure. Additional procedural detail from previous interventions was not captured by NSQIP, which subsequently did not allow us to determine the timing or indication of the prior procedure. Patients without any history of ipsilateral revascularization procedures were designated as undergoing primary bypass. Baseline and intraoperative characteristics, as well as 30-day postoperative outcomes were compared between patients undergoing primary and secondary bypass. In a subgroup analysis among patients undergoing secondary bypass, results were stratified according to the type of prior ipsilateral procedures (endovascular vs. bypass).

Clinical and outcome variables

Baseline characteristics included demographics, comorbidities, and pre-procedural medication. Age was evaluated as a continuous variable; however, all patients 90 years of age or older are recorded as 90+ by NSQIP to prevent individual patient identification. Antiplatelet medication preoperatively was considered when one of the following agents was documented: Aspirin, Clopidogrel, Eptifibatide, or Aggrenox. Intraoperative details analyzed included: procedure type, type of graft/conduit, and procedure time. Type of conduit was grouped by NSQIP into single segment greater saphenous vein (without documentation of an ipsilateral or contralateral harvested vein), or prosthetic or spliced/composite vein conduit (basilic, cephalic, or lesser saphenous vein). Concurrent suprainguinal procedures were identified with corresponding CPT codes (Supplementary table I). Since concurrent procedures did not affect outcomes when forced into the multivariable models or removed from the overall cohort, we did not exclude these patients from the analysis. Postoperative outcomes including 30-day mortality and adverse events were evaluated. Major adverse limb event (MALE), a composite variable endorsed by the Society for Vascular Surgery's Objective Performance Goals,[16] was defined as major amputation (below-knee or more proximal) or major reintervention (new or revision lower extremity bypass operation, jump/interposition graft revision, bypass graft thrombectomy/thrombolysis) of the index limb. Major adverse cardiovascular event (MACE) included death from any cause, myocardial infarction, or stroke. Wound infection included superficial, deep, or organ space surgical site infections. Postoperative renal insufficiency was defined as a serum creatinine concentration

Table I. Baseline characteristics and comorbidities in patients undergoing primary bypass versus bypass with prior revascularization

	CLTI									
	Primary Bypass *(N=3102)*		**Secondary Bypass** *(N=2031)*		**P-value**	**Prior Bypass** *(N=1145)*		**Prior Endovascular** *(N=886)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Male gender	1954	(63)	1230	(61)	.08	715	(62)	515	(58)	**.048**
Age – years (mean ± SD)	68.8 (12)		67.6 (11)		**<.001**	67.4 (11)		67.9 (12)		.29
Current smoker	1208	(39)	836	(41)	.11	471	(41)	365	(41)	.98
BMI > 30	894	(30)	584	(29)	.92	316	(28)	268	(31)	.27
Race					**<.001**					**<.01**
White	1890	(61)	1372	(68)		806	(70)	566	(64)	
Non-White	878	(28)	462	(23)		232	(20)	230	(26)	
Unknown/not reported	334	(11)	197	(9.7)		107	(9.3)	90	(10)	
Symptom status					**<.001**					**.001**
Rest pain	1158	(37)	987	(49)		594	(52)	393	(44)	
Tissue loss	1944	(63)	1044	(51)		551	(48)	493	(56)	
Hypertension	2584	(83)	1731	(85)	.07	957	(84)	774	(87)	**.02**
Diabetes	1592	(51)	1005	(50)	.20	515	(45)	490	(55)	**<.001**
CHF	108	(3.5)	68	(3.3)	.80	36	(3.1)	32	(3.6)	.56
Renal Insufficiency	846	(28)	453	(23)	**<.001**	233	(21)	220	(25)	**.02**
Dialysis	281	(9.1)	122	(6)	**<.001**	56	(4.9)	66	(7.4)	**.02**
COPD	392	(13)	269	(13)	.53	168	(15)	101	(11)	**.03**
Pre-procedural medication										
Antiplatelet	2354	(76)	1755	(87)	**<.001**	983	(86)	772	(87)	.45
Statin	2061	(67)	1497	(74)	**<.001**	848	(74)	649	(73)	.69
Beta blocker	1931	(63)	1322	(65)	.08	744	(65)	578	(65)	.97

	Claudication									
	Primary Bypass *(N=1438)*		**Secondary Bypass** *(N=731)*		**P-value**	**Prior Bypass** *(N=391)*		**Prior Endovascular** *(N=340)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Male gender	990	(69)	521	(71)	.25	278	(71)	243	(72)	.91
Age – years (mean ± SD)	65.9 (10)		66.0 (10)		.82	66.1 (10)		65.9 (10)		.72
Current smoker	662	(46)	314	(43)	.17	165	(42)	149	(44)	.66
BMI > 30	476	(33)	237	(33)	.75	121	(31)	116	(34)	.37
Race					.85					**.04**
White	1103	(77)	554	(76)		307	(79)	247	(73)	
Non-White	213	(15)	115	(16)		49	(13)	66	(19)	
Unknown/not reported	122	(8.5)	62	(8.5)		45	(9)	27	(7.9)	
Hypertension	1141	(79)	590	(81)	.45	320	(82)	270	(79)	.41
Diabetes	460	(32)	260	(36)	.09	130	(33)	130	(38)	.16
CHF	17	(1.2)	8	(1.1)	.86	7	(1.8)	1	(0.3)	.07
Renal Insufficiency	208	(15)	108	(15)	.91	63	(16)	45	(14)	.29
Dialysis	20	(1.4)	7	(1)	.39	5	(1.3)	2	(0.6)	.46
COPD	207	(14)	97	(13)	.48	51	(13)	46	(14)	.85
Pre-procedural medication										
Antiplatelet	1150	(81)	639	(88)	**<.001**	338	(87)	301	(89)	.28
Statin	962	(67)	558	(77)	**<.001**	298	(77)	260	(77)	.97
Beta blocker	755	(53)	414	(57)	.08	223	(58)	191	(57)	.79

SD: standard deviation, *BMI*: body mass index, *CHF*: congestive heart failure, *COPD*: chronic obstructive pulmonary disease

> 2 mg/dL and/or the need for dialysis. A respiratory complication was considered when one of the following was documented: pneumonia, unplanned reintubation, or ventilator requirement > 48 hours. Bleeding was defined as any transfusion or secondary procedure with the indication of bleeding. Variable definitions were provided by NSQIP prior to data collection and thus were not modifiable (user guides for targeted and non-targeted variables available at: https://www.facs.org/quality-programs/acs-nsqip/program-specifics/participant-use). Unplanned readmissions and reoperations to any hospital within 30 days of the index bypass were also collected. Since more granular NSQIP data on readmission and reoperation became available from 2012, analysis of indications for related unplanned readmission and reoperation were restricted to 2012-2014. Indications for related unplanned readmissions were captured by a NSQIP variable listing specific complications or International Classification of Disease (ICD-9) codes (Supplementary table II). CPT codes were used to group indications for related unplanned reoperations (Supplementary table III). Limb-related reoperations or readmissions were defined as all reinterventions or rehospitalizations respectively related to the ipsilateral index limb or procedure.

Statistical analysis

Differences between primary and secondary bypass, as well as between prior endovascular intervention and prior bypass, were evaluated using Pearson's χ^2 and Fisher's exact test for categorical variables, and Student's t-test and Mann Whitney U test for continuous variables, where appropriate. All analyses were stratified by symptom status. Multivariable logistic regression was used to establish the independent association between prior ipsilateral procedures and perioperative outcomes. Purposeful selection of covariates was performed to populate the multivariable models, with a cutoff point of $P < .10$ for inclusion of covariates on univariate screen.[17] Separate models were constructed for each perioperative outcome. All tests were two-sided and a value of $P < .05$ was considered significant. Statistical analysis was performed using SPSS Statistics 23 (IBM Corp, Armonk, NY).

RESULTS

A total of 7,302 patients were included, with 4,540 (62%) undergoing primary bypass (68% of these were performed for CLTI) and 2,762 (38%) undergoing secondary bypass (74% for CLTI). Among patients undergoing secondary bypass, 1,536 (56%) had prior ipsilateral bypass (75% for CLTI) and 1,226 (44%) had prior ipsilateral endovascular intervention (72% for CLTI).

Baseline characteristics

Baseline characteristics are detailed in Table I. Compared to CLTI patients undergoing primary bypass, those undergoing secondary bypass were younger (67.6 vs. 68.8 years, $P < .001$), more likely to be white (68% vs. 61%, $P < .001$) and less frequently had tissue

loss (51% vs. 63%, P < .001). In terms of comorbidities, CLTI patients with prior revascularization had less renal insufficiency (23% vs. 28%, P < .001) and were less often on dialysis preoperatively (6% vs. 9.1%, P < .001). Finally, secondary bypass patients were more likely to be treated with an antiplatelet agent (87% vs. 76%, P < .001) or statin preoperatively (74% vs. 67%, P < .001).

Among patients with claudication, demographics and comorbidities were similar. However, patients with prior revascularization were more likely to be treated with an antiplatelet agent (88% vs. 81%, P < .001) or statin preoperatively (77% vs. 67%, P < .001).

Operative details

Operative details are listed in Table II. Secondary bypass was associated with a significantly longer procedure time compared to primary bypass in patients with CLTI (240 min vs. 223 min, P < .001). Among patients with CLTI undergoing secondary bypass, saphenous vein conduits were less frequently used (54% vs. 64%, P < .001) and prosthetic or spliced/composite vein conduits were more commonly used (46% vs. 36%, P < .001). In addition, femoral-tibial/pedal bypass procedures were performed more often in CLTI patients with prior revascularization (39% vs. 32%, P < .001), whereas femoropopliteal bypass (51% vs. 56%, P < .001) and popliteal-tibial/pedal bypass (9.9% vs. 12%, P < .01) were less common in those undergoing secondary bypass. Concurrent suprainguinal procedures were evenly distributed between primary and secondary bypass.

Similar to patients with CLTI, those with claudication undergoing secondary bypass had longer procedure times (207 min vs. 187 min, P < .001), were less frequently revascularized with a saphenous vein conduit (53% vs. 59%, P = .02) and more often with prosthetic or spliced/composite vein conduits (47% vs. 41%, P = .02). Finally, those undergoing a second revascularization were more likely to have a femoral-tibial/pedal bypass (22% vs. 14%, P < .001), and less likely to undergo a femoropopliteal bypass (72% vs. 81%, P < .001).

Postoperative outcomes

Among CLTI patients undergoing secondary compared to primary bypass, similar rates of 30-day mortality were observed (1.7% vs. 2.2%, P = .22; Table III). Secondary bypass was associated with various adverse events, including MALE (9.8% vs. 7.4%, P < .01), major reintervention (6.5% vs. 4.7%, P < .01), bleeding leading to transfusion or secondary procedure (22% vs. 18%, P < .001), wound infection (9.5% vs. 7.8%, P = .04), and untreated loss of patency (3.6% vs. 2.1%, P < .01). In addition, CLTI patients with prior revascularization were more likely to be discharged to home (72% vs. 67%, P < .001).

Among claudication patients, 30-day mortality did not differ between secondary and primary bypass (0.4% vs. 0.6%, P = .76). Patients with prior revascularization had significantly more MALE (5.2% vs. 2.5%, P < .01), major reintervention (4.4% vs. 2.2%, P < .01), bleeding leading to transfusion or secondary procedure (12% vs. 6.7%, P < .001), unplanned reoperation (9.8% vs. 6.9%, P = .02), and longer hospital stay (4 days vs. 3 days, P < .01).

Table II. Procedure details in patients undergoing primary bypass versus bypass with prior revascularization

	CLTI									
	Primary Bypass *(N=3102)*		**Secondary Bypass** *(N=2031)*		**P-value**	**Prior Bypass** *(N=1145)*		**Prior Endovascular** *(N=886)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Type procedure					**<.001**					**.001**
Femoropopliteal bypass	1732	(56)	1029	(51)		559	(49)	470	(53)	
Femoral-tibial/pedal bypass	987	(32)	800	(39)		489	(43)	311	(35)	
Popliteal-tibial/pedal bypass	383	(12)	202	(9.9)		97	(8.5)	105	(12)	
Graft type					**<.001**					**<.001**
Saphenous vein	1975	(64)	1088	(54)		518	(45)	570	(64)	
Prosthetic/spliced/ composite vein	1127	(36)	943	(46)		627	(55)	316	(36)	
Concurrent suprainguinal procedure	121	(3.9)	77	(3.8)	.84	41	(3.6)	36	(4.1)	.57
Procedure time – (min ± IQR)	223 (171-296)		240 (179-325)		**<.001**	234 (178-331)		246 (183-318)		.38
Femoropopliteal bypass	204 (153-173)		218 (166-301)		**<.001**	213 (164-296)		229 (170-305)		.12
Femoral-tibial/pedal bypass	251 (193-322)		266 (199-345)		**<.01**	262 (193-352)		269 (204-338)		.55
Popliteal-tibial/pedal bypass	244 (197-307)		268 (202-337)		**.02**	270 (203-343)		264 (199-325)		.42
	Claudication									
	Primary Bypass *(N=1438)*		**Secondary Bypass** *(N=731)*		**P-value**	**Prior Bypass** *(N=391)*		**Prior Endovascular** *(N=340)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Type procedure					**<.001**					**.001**
Femoropopliteal bypass	1168	(81)	526	(72)		258	(66)	268	(79)	
Femoral-tibial/pedal bypass	205	(14)	164	(22)		106	(27)	58	(17)	
Popliteal-tibial/pedal bypass	65	(4.5)	41	(5.6)		27	(6.9)	14	(4.1)	
Graft type					**.02**					**.01**
Saphenous vein	841	(59)	389	(53)		191	(49)	198	(58)	
Prosthetic/spliced/ composite vein	597	(41)	342	(47)		200	(51)	142	(42)	
Concurrent suprainguinal procedure	47	(3.3)	27	(3.7)	.61	15	(3.8)	12	(3.5)	.83
Procedure time – (min ± IQR)	187 (135-253)		207 (158-282)		**<.001**	210 (163-281)		202 (153-283)		.35
Femoropopliteal bypass	175 (129-241)		198 (149-273)		**<.001**	198 (148-273)		196 (150-274)		.88
Femoral-tibial/pedal bypass	238 (186-302)		228 (187-314)		.83	232 (191-307)		222 (178-334)		.53
Popliteal-tibial/pedal bypass	199 (165-279)		240 (164-328)		.16	230 (141-314)		274 (197-368)		.38

min: minutes; *IQR*: interquartile range

Table III. Perioperative outcomes in patients undergoing primary bypass versus bypass with prior revascularization

	CLTI									
	Primary Bypass *(N=3102)*		**Secondary Bypass** *(N=2031)*		**P-value**	**Prior Bypass** *(N=1145)*		**Prior Endovascular** *(N=886)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Mortality	67	(2.2)	34	(1.7)	.22	14	(1.2)	20	(2.3)	.07
MALE	231	(7.4)	200	(9.8)	**<.01**	129	(11)	71	(8)	**.02**
Major amputation	116	(3.7)	88	(4.3)	.29	52	(4.5)	36	(4.1)	.60
Major reintervention	147	(4.7)	132	(6.5)	**<.01**	89	(7.8)	43	(4.9)	**<.01**
MACE	155	(5)	103	(5.1)	.91	49	(4.3)	54	(6.1)	.06
Wound infection	242	(7.8)	192	(9.5)	**.04**	84	(7.3)	108	(12)	**<.001**
Wound dehiscence	63	(2)	36	(1.8)	.51	22	(1.9)	14	(1.6)	.56
Sepsis	48	(1.5)	33	(1.6)	.83	15	(1.3)	18	(2)	.20
Bleeding	563	(18)	453	(22)	**<.001**	249	(22)	204	(23)	.49
Creatinine >2 mg/dL	42	(1.4)	25	(1.2)	.70	15	(1.3)	10	(1.1)	.71
Requiring dialysis	21	(0.7)	10	(0.5)	.40	7	(0.6)	3	(0.3)	.53
Respiratory complications	110	(3.5)	56	(2.8)	.12	31	(2.7)	25	(2.8)	.88
Urinary tract infection	44	(1.4)	33	(1.6)	.55	13	(1.1)	20	(2.3)	**.047**
Unplanned reoperation	537	(17)	389	(19)	.09	209	(18)	180	(20)	.24
Untreated loss of patency	65	(2.1)	73	(3.6)	**.001**	50	(4.4)	23	(2.6)	**.03**
Length of hospital stay – (d ± IQR)	7 (4-12)		7 (4-12)		.91	7 (4-11)		7 (4-12)		.56
Discharge to home	1923	(67)	1374	(72)	**<.001**	814	(75)	560	(68)	**.001**
Unplanned readmission	559	(18)	367	(18)	.96	199	(17)	168	(19)	.36
	Claudication									
	Primary Bypass *(N=1438)*		**Secondary Bypass** *(N=731)*		**P-value**	**Prior Bypass** *(N=391)*		**Prior Endovascular** *(N=340)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Mortality	9	(0.6)	3	(0.4)	.76	3	(0.8)	0	(0)	.25
MALE	36	(2.5)	38	(5.2)	**.001**	25	(6.4)	13	(3.8)	.12
Major amputation	7	(0.5)	7	(1)	.20	3	(0.8)	4	(1.2)	.71
Major reintervention	32	(2.2)	32	(4.4)	**<.01**	22	(5.6)	10	(2.9)	.08
MACE	27	(1.9)	17	(2.3)	.48	9	(2.3)	8	(2.4)	.96
Wound infection	101	(7)	52	(7.1)	.94	21	(5.4)	31	(9.1)	**.049**
Wound dehiscence	16	(1.1)	9	(1.2)	.81	8	(2)	1	(0.3)	**.04**
Sepsis	14	(1)	5	(0.7)	.49	4	(1)	1	(0.3)	.38
Bleeding	97	(6.7)	84	(12)	**<.001**	49	(13)	35	(10)	.34
Creatinine >2 mg/dL	9	(0.6)	4	(0.5)	>.99	3	(0.8)	1	(0.3)	.63
Requiring dialysis	3	(0.2)	3	(0.4)	.41	2	(0.5)	1	(0.3)	>.99
Respiratory complications	15	(1)	10	(1.4)	.50	6	(1.5)	4	(1.2)	.76
Urinary tract infection	8	(0.6)	5	(0.7)	.72	1	(0.3)	4	(1.2)	.19
Unplanned reoperation	99	(6.9)	72	(9.8)	**.02**	43	(11)	29	(8.5)	.26
Untreated loss of patency	11	(0.8)	12	(1.6)	.06	10	(2.6)	2	(0.6)	**.04**
Length of hospital stay – (d ± IQR)	3 (2-5)		4 (3-6)		**<.01**	4 (2-6)		4 (3-5)		.91
Discharge to home	1298	(91)	658	(90)	.63	353	(90)	305	(90)	.98
Unplanned readmission	141	(9.8)	76	(10)	.66	45	(12)	31	(9.1)	.29

MALE: major adverse limb event; *MACE*: major adverse cardiovascular event; *d:* days; *IQR*: interquartile range

In a subgroup analysis of secondary bypass patients, we compared outcomes of those with prior bypass to those with prior endovascular intervention. Among CLTI patients with prior bypass, there was a trend towards lower mortality, although significance was not achieved (1.2% vs. 2.3%, P = .07). Similarly, no difference in mortality was observed in patients with claudication between prior bypass and endovascular intervention (0.8% vs. 0%, P = .25). Prior endovascular intervention was associated with more wound infections for CLTI (12% vs. 7.8%, P < .001) and claudication patients (9.1% vs. 5.4%, P = .049). In addition, revascularization with saphenous vein conduits was more common in patients with prior endovascular intervention compared to those with prior bypass in CLTI (64% vs. 45%, P < .001) and claudication (58% vs. 49%, P = .01). Patients with CLTI and a prior bypass had higher rates of MALE (11% vs. 8%, P = .02), major reintervention (7.8% vs. 4.9%, P < .01), and were more likely to be discharged to home (75% vs. 68%, P < .01) than those with prior endovascular intervention. Untreated loss of patency occurred more frequently in patients undergoing secondary bypass after prior bypass with CLTI (4.4% vs. 2.6%, P = .03) and claudication (2.6% vs. 0.6%, P = .04) compared to those with prior endovascular intervention.

Reoperations and readmissions

Unplanned reoperations were more common in patients undergoing secondary compared to primary bypass for both CLTI (15% vs. 13%, P = .01) and claudication (9% vs. 5.6%, P < .01; Table IV). Unplanned reoperations were primarily limb-related, with more open and endovascular revascularizations in CLTI patients undergoing secondary bypass (7.1% vs. 4.7%, P = .001), whereas major and minor amputations were higher in those undergoing bypass with prior endovascular intervention (4.9% vs. 3.0%, P = .04). There was no significant difference in reoperation rates between claudication patients with a prior bypass and a prior endovascular intervention.

Comparable rates of unplanned readmissions were observed between primary and secondary bypass for CLTI (13% vs. 14%, P = .25) and claudication (7.6% vs. 8.7%, P = .38; Table V). Additionally, the most common reason for readmission was infection in patients with CLTI (55%) and claudication (54%).

Multivariable analysis

Primary vs. secondary bypass

In adjusted analysis (Table VI), secondary bypass was found to be an independent predictor of MALE for both CLTI (OR: 1.4, 95% CI: 1.1-1.7) and claudication patients (OR: 2.1, 95% CI: 1.3-3.5). Prior revascularization in CLTI patients was also associated with major amputation (OR: 1.3, 95% CI: 1.01-1.8), major reintervention (OR: 1.4, 95% CI: 1.1-1.8), bleeding leading to transfusion or secondary procedure (OR: 1.4, 95% CI: 1.2-1.6), untreated loss of patency (OR: 1.9, 95% CI: 1.3-2.7), and unplanned reoperation (OR: 1.2, 95% CI: 1.02-1.4). In claudication patients, prior revascularization proved to be an important risk factor for major reintervention (OR: 2.1, 95% CI: 1.3-3.5), bleeding leading to transfusion or secondary procedure (OR: 1.7, 95% CI: 1.3-2.4), and unplanned reoperation (OR: 1.6, 95% CI: 1.1-2.1).

Table IV. Unplanned reoperations and indications in patients undergoing primary bypass versus bypass with prior revascularization (analysis restricted to 2012-2014)

	CLTI									
	Primary Bypass *(N=2854)*		**Secondary Bypass** *(N=1801)*		**P-value**	**Prior Bypass** *(N=1028)*		**Prior Endovascular** *(N=773)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Related to principle procedure					**.04**					.39
1 Unplanned reoperation	292	(10)	218	(12)		116	(11)	102	(13)	
>1 Unplanned reoperation	69	(2.4)	57	(3.2)		35	(3.4)	22	(2.8)	
Indications of related reoperations										
Limb-related reoperations	343	(12)	261	(15)	**.01**	143	(14)	118	(15)	.42
Incision & drainage/debridement	124	(4.3)	82	(4.6)	.74	40	(3.9)	42	(5.4)	.12
Major/minor amputation	103	(3.6)	69	(3.8)	.70	31	(3)	38	(4.9)	**.04**
Open or endovascular revascularization	125	(4.7)	128	(7.1)	**.001**	82	(8)	46	(6)	.10
Other vascular	11	(0.4)	8	(0.4)	.76	5	(0.5)	3	(0.4)	>.99
Other reoperations	14	(0.5)	13	(0.7)	.31	7	(0.7)	6	(0.8)	.81
	Claudication									
	Primary Bypass *(N=1302)*		**Secondary Bypass** *(N=652)*		**P-value**	**Prior Bypass** *(N=346)*		**Prior Endovascular** *(N=306)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Related to principle procedure					**.01**					.57
1 Unplanned reoperation	64	(4.9)	48	(7.4)		29	(8.4)	19	(6.2)	
>1 Unplanned reoperation	9	(0.7)	11	(1.7)		6	(1.7)	5	(1.6)	
Indications of related reoperations										
Limb-related reoperations	68	(5.2)	49	(7.5)	**.04**	28	(8.1)	21	(6.9)	.55
Incision & drainage/debridement	29	(2.2)	22	(3.4)	.13	10	(2.9)	12	(3.9)	.47
Major/minor amputation	7	(0.5)	6	(0.9)	.33	3	(0.9)	3	(1)	>.99
Open or endovascular revascularization	33	(2.5)	23	(3.5)	.22	15	(4.3)	8	(2.6)	.24
Other vascular	1	(0.1)	4	(0.6)	.05	2	(0.6)	2	(0.7)	>.99
Other reoperations	7	(0.5)	8	(1.2)	.10	6	(1.7)	2	(0.7)	.29

Table V. Unplanned readmissions and indications in patients undergoing primary bypass versus bypass with prior revascularization (analysis restricted to 2012-2014)

	CLTI									
	Primary Bypass *(N=2854)*		**Secondary Bypass** *(N=1801)*		**P-value**	**Prior Bypass** *(N=1028)*		**Prior Endovascular** *(N=773)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Related to principle procedure					.44					.22
1 Unplanned readmission	359	(13)	245	(14)		131	(13)	114	(15)	
>1 Unplanned readmission	10	(0.4)	9	(0.5)		7	(0.7)	2	(0.3)	
Indications of related readmissions										
Limb-related readmissions	299	(11)	218	(12)	.09	123	(12)	95	(12)	.83
Infection	212	(7.4)	132	(7.3)	.90	70	(6.8)	62	(8)	.33
Non-healing/open surgical wound	24	(0.8)	17	(0.9)	.71	12	(1.2)	5	(0.6)	.26
Restenosis/occlusion/ complication bypass	25	(0.9)	33	(1.8)	**<.01**	20	(1.9)	13	(1.7)	.68
Thromboembolic events	11	(0.4)	3	(0.2)	.27	2	(0.2)	1	(0.1)	>.99
Hemorrhage/seroma	19	(0.7)	23	(1.3)	**.03**	12	(1.2)	11	(1.4)	.63
Pain complications	5	(0.2)	7	(0.4)	.16	5	(0.5)	2	(0.3)	.71
Other complications	6	(0.2)	8	(0.4)	.16	6	(0.6)	2	(0.3)	.48
Other readmissions	60	(2.1)	34	(1.9)	.61	16	(1.6)	18	(2.3)	.23
	Claudication									
	Primary Bypass *(N=1302)*		**Secondary Bypass** *(N=652)*		**P-value**	**Prior Bypass** *(N=346)*		**Prior Endovascular** *(N=306)*		**P-value**
	N	*%*	*N*	*%*		*N*	*%*	*N*	*%*	
Related to principle procedure					.60					.31
1 Unplanned readmission	96	(7.4)	56	(8.6)		34	(9.8)	22	(7.2)	
>1 Unplanned readmission	3	(0.2)	1	(0.2)		1	(0.3)	0	(0)	
Indications of related readmissions										
Limb-related readmissions	81	(6.2)	46	(7.1)	.48	28	(8.1)	18	(5.9)	.27
Infection	57	(4.4)	27	(4.1)	.81	14	(4)	13	(4.2)	.90
Non-healing/open surgical wound	9	(0.7)	5	(0.8)	.85	4	(1.2)	1	(0.3)	.38
Restenosis/occlusion/ complication bypass	2	(0.2)	4	(0.6)	.10	3	(0.9)	1	(0.3)	.63
Thromboembolic events	1	(0.1)	2	(0.3)	.26	2	(0.6)	0	(0)	.50
Hemorrhage/seroma	4	(0.3)	6	(0.9)	.09	5	(1.4)	1	(0.3)	.22
Pain complications	5	(0.4)	1	(0.2)	.67	0	(0)	1	(0.3)	.47
Other complications	3	(0.2)	1	(0.2)	>.99	0	(0)	1	(0.3)	.47
Other readmissions	17	(1.3)	9	(1.4)	.89	7	(2)	2	(0.7)	.18

Prior bypass surgery vs. prior endovascular intervention

To assess associations with adverse events and prior procedure type, an additional subgroup analysis in the secondary bypass cohort was performed. Among patients undergoing secondary bypass for CLTI, prior bypass was associated with MALE (OR: 1.4, 95% CI: 1.03-1.9) and major reintervention (OR: 1.5, 95% CI: 1.03-2.2) compared to prior endovascular intervention. After adjusting for several covariates (e.g. graft type, diabetes, tissue loss), CLTI patients with a prior bypass had a decreased risk of wound infection (OR: 0.6, 95% CI: 0.4-0.8) compared to those with a prior endovascular intervention. A similar trend was seen in patients with claudication but this did not reach significance (OR: 0.6, 95% CI: 0.3-1.05). To further evaluate the effect of prior endovascular treatment on wound infection, we compared primary bypass to secondary bypass with prior endovascular intervention only, and still found a higher risk of wound infection following prior endovascular treatment (12% vs. 7.8%; OR: 1.5, 95% CI: 1.2-2.0; Supplementary table IV).

Table VI A. Adjusted associations between prior revascularization and perioperative outcomes in chronic limb-threatening ischemia (CLTI) patients

	CLTI					
	Secondary bypass vs. primary bypass			**Prior bypass vs. prior endovascular**		
	OR	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
Mortality	0.9	0.6-1.4	.62	0.6	0.3-1.2	.13
MALE [a]	1.4	1.1-1.7	**<.01**	1.4	1.03-1.9	**.03**
Major amputation [b]	1.3	1.01-1.8	**.049**	1.1	0.7-1.8	.54
Major reintervention [c]	1.4	1.1-1.8	**.02**	1.5	1.03-2.2	**.04**
MACE	1.1	0.8-1.4	.68	0.7	0.5-1.1	.16
Wound infection [d]	1.2	0.96-1.5	.12	0.6	0.4-0.8	**.001**
Bleeding (leading to transfusion or sec. procedure) [e]	1.4	1.2-1.6	**<.001**	1.0	0.8-1.2	.98
Untreated loss of patency [f]	1.9	1.3-2.7	**.001**	1.5	0.9-2.5	.12
Unplanned reoperation [g]	1.2	1.02-1.4	**.03**	0.9	0.7-1.2	.59

MALE: major adverse limb event, *MACE:* major adverse cardiovascular event

All adjusted for: age, sex, tissue loss, and type of procedure. Additionally adjusted for: [a] current smoking, obesity, race, hypertension, diabetes mellitus, preoperative antiplatelet use, graft type; [b] race, diabetes mellitus, renal insufficiency, dialysis, preoperative antiplatelet use; [c] current smoking, obesity, hypertension, diabetes mellitus, preoperative antiplatelet use, graft type; [d] obesity, diabetes mellitus, preoperative statin use, graft type; [e] race, congestive heart failure, renal insufficiency, dialysis, preoperative antiplatelet/ statin/ beta blocker use; [f] obesity, renal insufficiency, preoperative antiplatelet use, graft type; [g] obesity, race, diabetes mellitus, dialysis, preoperative antiplatelet/ beta blocker use, graft type

Table VI B. Adjusted associations between prior revascularization and perioperative outcomes in patients with claudication

	Claudication					
	Secondary bypass vs. primary bypass			Prior bypass vs. prior endovascular		
	OR	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
Mortality *	-	-	-	-	-	-
MALE [a]	2.1	1.3-3.5	**<.01**	1.7	0.8-3.4	.14
Major amputation *	-	-	-	-	-	-
Major reintervention [b]	2.1	1.3-3.5	**<.01**	2.1	0.9-4.5	.07
MACE	1.1	0.6-2.1	.66	0.9	0.3-2.3	.80
Wound infection	1.0	0.7-1.5	.81	0.6	0.3-1.05	.07
Bleeding (leading to transfusion or sec. procedure) [c]	1.7	1.3-2.4	**.001**	1.1	0.7-1.8	.71
Untreated loss of patency	2.2	0.9-5.3	.07	3.7	0.8-17.4	.10
Unplanned reoperation [d]	1.6	1.1-2.1	**<.01**	1.2	0.7-2.0	.41

MALE: major adverse limb event, *MACE:* major adverse cardiovascular event
* Too few events
All adjusted for: age, sex, and type of procedure. Additionally adjusted for: [a] race, diabetes mellitus, renal insufficiency, preoperative antiplatelet use, graft type; [b] diabetes mellitus, renal insufficiency, graft type; [c] obesity, race, hypertension, preoperative antiplatelet/ statin use; [d] diabetes mellitus, renal insufficiency, dialysis, preoperative antiplatelet use

DISCUSSION

This study demonstrates increased risk of adverse perioperative outcomes in patients undergoing a secondary compared to primary bypass. Patients undergoing secondary bypass for CLTI or claudication were at increased risk of 30-day MALE, major reintervention, and unplanned reoperation. Subgroup analysis found that secondary bypass with prior endovascular intervention was a prominent predictor of wound infections, whereas 30-day major reintervention was more commonly performed following bypass after prior bypass.

In 2005, the randomized BASIL trial compared endovascular- or bypass-first strategy in limb ischemia patients and found similar morbidity and mortality rates up to two years after surgery.[18] Although the BASIL trial has been criticized for multiple shortcomings in study design, further analysis demonstrated that bypass surgery was associated with decreased mortality from 2 years onward compared to endovascular intervention alone (HR: 0.61, 95% CI: 0.50-0.75).[19] Therefore, current ACC/AHA guidelines recommend that bypass surgery be preferentially performed over endovascular intervention in CLTI patients with a life expectancy of greater than two years.[20] Nonetheless, a bypass-first approach is not widely accepted as the optimal treatment option and results of the BEST-CLI trial are still pending.[21] Additionally, many institutions have adopted an endovascular-first approach

in PAD patients because it is less invasive and therefore associated with less perioperative risk, although perioperative mortality is similar. Since management of PAD does not end with the first intervention in many cases, several studies were undertaken to assess whether failed initial treatment affects the outcome of subsequent revascularization.[6-12] In the BASIL trial, further analysis showed that patients undergoing a secondary bypass with prior endovascular intervention had a notable one-year failure rate (defined as death, major amputation, recurrent symptoms, or reintervention) of 54% compared to 70% in the bypass group with prior bypass.[22]

In 2011, Nolan et al. studied the Vascular Study Group of New England (VSGNE) database and demonstrated that, for 1,880 bypass surgeries performed for CLTI, both prior endovascular intervention and prior bypass surgery were independently associated with one-year amputation and graft occlusion.[9] However, there were no demonstrable differences in 30-day outcomes.

Subsequently, Jones et al. studied an expanded cohort of 3,504 patients undergoing bypass surgery from the VSGNE and, using inverse probability weighted analyses, demonstrated inferior one-year outcomes associated with secondary compared to primary bypass in CLTI patients, including MALE-free survival (55% vs. 63%, $P < .01$) and reintervention or amputation-free survival (53% vs. 60%, $P < .01$).[6] Interestingly, adverse events following secondary bypass were not affected by the type of primary treatment, either endovascular or bypass. In the perioperative period, patients undergoing secondary bypass were also more likely to return to the operating room for graft thrombosis during their index hospitalization and more frequently had graft occlusion at discharge. Despite these findings, no differences were found for secondary bypass patients with regards to other major in-hospital adverse events, including mortality, myocardial infarction, or ipsilateral amputation, regardless of indication. In accordance with these findings, our data also indicated that patients undergoing secondary bypass more often had 30-day adverse limb events, with both ipsilateral major amputation and reintervention as driving factors in CLTI patients, whereas major reintervention alone was the primary driver in those with claudication.

Several other studies reported short-term outcomes. In comparison to primary bypass treatment, Uhl et al. found no association between prior endovascular intervention and 30-day mortality, graft failure, or major amputation.[11] In addition, Santo el al. determined that prior endovascular intervention was not associated with 30-day mortality or myocardial infarction and was not a predictor for overall wound complication compared to primary bypass.[12] Although we showed similar short-term mortality rates, our data indicated that prior endovascular intervention was associated with a 1.5-fold increased risk of wound infection compared to primary treatment as well as secondary bypass following prior bypass surgery after adjusting for multiple confounders such as graft type, diabetes, and tissue loss. The published rate of surgical site infections after bypass has varied, with a reported incidence of 5-23%; however, no recent studies identified prior revascularization as a risk factor.[18,23,24] This could be related to the more frequent use of saphenous veins as conduits

compared to prosthetic or arm veins and the increased likelihood of wound infections associated with ipsilateral autogenous vein harvesting. However, we still observed an increased risk of wound infections comparing prior endovascular intervention to primary bypass, while the proportion of revascularization with saphenous vein conduits between these groups was similar. Unmeasured confounding variables such as ipsilateral vs. contralateral vein harvest, length and number of incisions, and basilic/cephalic vs. saphenous vein conduits may impact this study and cannot be accounted for. In addition, the type and number of previous endovascular interventions were unfortunately not captured by NSQIP, and could therefore not be evaluated to better answer why prior endovascular intervention patients were at increased risk for wound infections.

The increased risks of adverse events following secondary revascularization may be explained by a more aggressive disease process. Patients who have already suffered failure of a primary procedure are likely to represent a selected group that is at greater general risk for treatment failure and other adverse outcomes. In addition, due to unfavorable anatomy and hampered inflow or runoff vessels caused by previous procedures, these patients may be predisposed to an increased risk of complication. We attempted to account for this with multivariable modeling; however, unmeasured indicators of more aggressive disease phenotype cannot be controlled for. This study does not attempt to shed light on the optimal primary treatment strategy, nor does it answer which secondary treatment strategy is superior. However, there are important clinical implications to this study. First, these short-term outcomes may provide clinicians valuable guidance in the selection and counseling of PAD patients. Furthermore, physicians should factor in the significant association of failed prior ipsilateral treatment with future interventions and recognize inferior outcomes of repeated procedures.

This study has several limitations. First, NSQIP has potential errors in coding and misreporting of data. Second, the lesion severity characteristics and extent of PAD, as well as explicit detail of the timing and procedural information from patients' previous interventions, are not available in this clinical registry. Prior procedures were taken directly from the patients' medical records by clinical reviewers; however, with these strict variable definitions we were unable to identify patients with both a prior endovascular and surgical revascularization. We believe that these patients were most likely coded as having had a prior bypass rather than a prior endovascular intervention, which should be factored in when considering these study results. This would most likely bias our outcomes towards the null and thus we feel that the observed differences are likely to be real and perhaps underestimated. The importance of disease severity and type of prior procedure, particularly single or multilevel treatment and placement of stents, has been confirmed in previous studies,[25,26] but NSQIP-variables lack this level of granularity. The clinical registry also lacks detail on incision type, graft configuration (in situ vs. transposed/reversed anatomically tunneled graft), and severity of tissue loss (ulcers vs. gangrene), all of which could have added further detail to our comparison. However, the strength of NSQIP is the large sample size and its national representation. Although we could determine that primary treatment

failure is associated with worsened outcomes of secondary bypass, we cannot establish causation given the retrospective nature of the study design. Finally, NSQIP captures follow-up data only up to 30 days. Therefore, we were unable to determine long-term outcomes such as graft patency and amputation-free survival in those patients with prior revascularization.

CONCLUSIONS

This study demonstrates that bypass surgery following prior ipsilateral revascularization is associated with increased 30-day major adverse limb events in both CLTI and claudication patients. Other adverse events included major reintervention, bleeding leading to transfusion or secondary procedure, and unplanned reoperation. Furthermore, this study shows that the type of prior procedure is associated with outcomes of secondary bypass. In particular, prior endovascular intervention proved an important predictor for wound infections, and prior bypass was associated with MALE and major reintervention. The present findings should be considered in patient selection and during operative planning, particularly since the proportion of patients undergoing multiple revascularization procedures is rising.

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SUPPLEMENTARY

Supplementary table I. Concurrent suprainguinal procedures identified with Current Procedural Terminology (CPT) codes

Concurrent suprainguinal procedures	**CPT Codes**
Bypass surgery	35521, 35533, 35537, 35538, 35539, 35540, 35621,35623, 35637, 35646, 35647, 35663, 35665
Endovascular intervention	37220 – 37223

Supplementary table II. Indications of related unplanned readmissions according to NSQIP complication variables and International Classification of Disease, ninth edition (ICD-9) codes

Indications unplanned readmissions	NSQIP variable listing specific complications	ICD-9 codes
Limb-related readmissions		
Infection	Superficial Incisional SSI	040.0, 440.23-24, 681.x, 682.x, 707.x, 730.x, 785.4, 996.62-69, 997.62, 998.5-6, 70.261
	Deep Incisional SSI	
	Organ/Space SSI	
Non-healing/open surgical wound	Wound Disruption	998.30-32, 998.83, 58.41
Restenosis/occlusion/complication bypass	DVT Requiring Therapy	444.x, 445.02, 453.x, 82.868
	Vein Thrombosis Requiring Therapy	
Thromboembolic events		440.2, 440.30-32, 447.x, 459.9, 996.1, 996.74, 70.601
Hemorrhage/seroma	Bleeding Requiring Transfusion (72h of surgery start time)	729.91-92, 998.1-13
Pain complications		338.x, 440.21-22, 443, 443.9, 729.5
Other complications		285.1, 440.4, 442.3, 443.29, 729.72, 895, 997.2, 49.73-75
Other readmissions		
	Myocardial Infarction	008.45, 04.0, 38.9, 53.1, 81.11, 250.x, 251.2. 276.x, 285.9, 289.81, 292.81, 336.1, 786.x,
	Sepsis	349.82, 401.1, 404.91, 410.71, 414.01, 428.x, 432.1, 435.9, 458.0, 478.75, 518.81,
	Septic Shock	530.21, 531, 535.51, 560.1, 564, 578.x, 599.7, 709.8, 712.26, 733.9 780.x, 782.3, 783.7,
	Pneumonia	787.01, 789.00-06, 790.29, 863.21, 965.09, 996.72, 997.49, 997.99, 998.89
	Pulmonary Embolism	
	Unplanned Intubation	
	Acute Renal Failure	
	Progressive Renal Insufficiency	
	Urinary Tract Infection	

SSI: surgical site infection, *DVT:* deep vein thrombosis, *h:* hour

Supplementary table III. Indications of related unplanned reoperations according to CPT codes

Indications unplanned reoperations	CPT codes
Limb-related reoperations	
Incision & drainage/debridement	10140-11000, 11040-11046, 26990, 27301, 27600-27604, 27894-28001, 97597-97605
Major/minor amputation	11752, 27590-27596, 27880-27888, 28112-28825
Open or endovascular revascularization	34201-34451, 35151, 35226-35286, 35302-36245, 36870-37186, 37201-37607, 37618-37735, 75710-75726
Other vascular	12020, 13160-15832, 29871, 75900,
Other reoperations	
	33512-34151, 34830, 35301, 36833, 37191, 37609, 37799, 4110, 92982, 4470
	10060-10120, 11004-11005, 11400, 13121, 20680-26910, 27236, 27335, 31600-32609, 38500-38760, 42180-61314

Supplementary table IV. Adjusted associations between prior revascularization procedure and perioperative outcomes (reference group: primary bypass)

	CLTI					
		Prior bypass surgery			**Prior endovascular intervention**	
	OR	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
MALE	1.6	1.3-2.0	**<.001**	1.1	0.9-1.5	.39
Major amputation	1.4	1.01-2.0	**.046**	1.2	0.8-1.8	.27
Major reintervention	1.6	1.2-2.1	**.001**	1.1	0.7-1.5	.74
Wound infection	0.9	0.7-1.2	.41	1.5	1.2-2.0	**.001**
Bleeding (leading to transfusion or sec. procedure)	1.4	1.2-1.7	**<.001**	1.4	1.1-1.7	**.001**
Untreated loss of patency	2.2	1.5-3.3	**<.001**	1.5	0.9-2.4	.13
Unplanned reoperation	1.2	0.96-1.4	.13	1.2	1.01-1.5	**.04**
	Claudication					
		Prior bypass surgery			**Prior endovascular intervention**	
	OR	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
MALE	2.7	1.5-4.6	**<.001**	1.6	0.8-3.1	.18
*Major amputation **	-	-	-	-	-	-
Major reintervention	2.8	1.6-5.0	**<.001**	1.4	0.7-2.8	.41
Wound infection	0.8	0.6-1.3	.34	1.4	0.9-2.1	.17
Bleeding (leading to transfusion or sec. procedure)	1.7	1.2-2.3	**<.01**	1.6	1.1-2.5	**.02**
Untreated loss of patency	3.3	1.3-8.1	**.01**	0.9	0.2-4.1	.87
Unplanned reoperation	1.7	1.2-2.5	**<.01**	1.4	0.9-2.2	.14

MALE: major adverse limb event
* Too few events

PART TWO

EXPERIMENTAL STUDIES

CHAPTER 9

INTRALUMINAL DELIVERY OF THROMBOSPONDIN-2 SIRNA INHIBITS THE VASCULAR RESPONSE TO INJURY IN A RAT CAROTID BALLOON ANGIOPLASTY MODEL

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ABSTRACT

In an effort to inhibit the response to vascular injury leading to intimal hyperplasia, this study investigated the *in vivo* efficacy of intraluminal delivery of thrombospondin-2 (TSP-2) small interfering RNA (siRNA). Common carotid artery (CCA) balloon angioplasty injury was performed in rats. Immediately following denudation, CCA was transfected intraluminally (15 minutes) with one of the following: polyethylenimine (PEI)+TSP-2 siRNA, saline, PEI only, or PEI+control siRNA. CCA was analyzed at 24 hours or 21 days using qRT-PCR and immunohistochemistry. TSP-2 gene and protein expression were significantly upregulated following endothelial denudation at 24 hours and 21 days compared to the contralateral untreated non-denuded CCA. Treatment with PEI+TSP-2 siRNA significantly suppressed TSP-2 gene expression (3.1-fold) at 24 hours and TSP-2 protein expression, cell proliferation, and collagen deposition up to 21 days. These changes could be attributed to changes in transforming growth factor-βeta (TGF-β) and matrix metalloproteinase-9 (MMP-9), the downstream effectors of TSP-2. TSP-2 knockdown induced anti-inflammatory M2 macrophage polarization at 21 days; however, did not significantly affect intima/media ratios. In conclusion, these data demonstrate effective siRNA transfection of the injured arterial wall and provide a clinically effective and translationally applicable therapeutic strategy involving non-viral siRNA delivery to ameliorate the response to vascular injury.

INTRODUCTION

Peripheral arterial disease (PAD) affects 8.5 million Americans over 40 years of age and is most commonly due to atherosclerosis, with patients often presenting with claudication, ischemic rest pain, or non-healing wounds.[1] PAD is associated with significant morbidity and mortality, and with an aging population, the incidence of patients with PAD is expected to rise. Endovascular and surgical revascularization procedures are often necessary to alleviate ischemic symptoms after failed medical treatment. While endovascular interventions are increasingly utilized, autologous vein grafts are preferred in select patients and associated with significantly lower long-term amputation rates and improved overall survival.[2,3] The durability of vascular interventions, however, is limited by the development of intimal hyperplasia (IH) leading to restenosis. IH is a result of a complex pathologic response to damaging stimuli such as injury, inflammation, or shear stress that triggers macrophage infiltration, as well as proliferation and migration of numerous cells to the intima.[4-6] As a result, delayed failure rates occur in approximately 30-35% of all vein grafts within the first postoperative year.[7] IH has been a target of experimental therapies in order to improve long-term patency rates. However, thus far no effective treatment has been developed to selectively target IH following vascular procedures.

To better understand the pathophysiology of graft failure, we have analyzed cell-specific and time-dependent genomic alterations to arterial injury. While inflammatory cytokines dominated the early response, genes that involve proliferation and extracellular remodeling were subsequently identified.[8] In prior studies, we found thrombospondin-2 (TSP-2) to be substantially upregulated in neointimal tissue of prosthetic grafts and identified it as a high profile target.[9] Although TSP-2 is associated with wound healing and angiogenesis, its distinct role in IH remains unclear.

As an extracellular matrix (ECM) protein, TSP-2 functions primarily as an inhibitor of angiogenesis and a modulator of cell-matrix interactions, yet it does not contribute to structural stability of the ECM. Interestingly, wounds of TSP-2 null mice exhibit highly vascularized granulation tissue and abnormal organization of collagen fibrils, resulting in accelerated wound healing and minimal scarring.[10-12] Since IH is a result of an excessive pathologic wound healing response to injury, gene knockout of TSP-2 may inhibit the initial alterations leading to failure of vascular interventions.

In prior studies using small interfering RNA (siRNA) we demonstrated effective transfection of human aortic smooth muscle cells, thereby silencing TSP-2 with consequent protein knockdown *in vitro* for up to 30 days.[9] Accordingly, the purpose of this study was to evaluate the efficacy of *in vivo* localized intraluminal delivery of siRNA to the arterial wall in a rat carotid artery balloon angioplasty injury model. The specific objectives were to confirm, in an *in vivo* model, effective delivery of siRNA with knockdown of TSP-2 gene transcription and protein translation and to analyze TSP-2-dependent pathways that may contribute to inflammation and IH.

MATERIALS AND METHODS

siRNA design and transfection agent

The rat specific TSP-2 and the non-targeting control siRNA were obtained from Ambion (Life Technologies, Grand Island, NY, USA). To mediate efficient siRNA delivery, polyethylenimine (*in vivo* jetPEI®, Polyplus, Strasbourg, France) was used as a transfection reagent according to the manufacturer's instructions. Information about siRNA is provided in Supplementary Table 1.

Animal model and surgical procedure

Protocols for this study were approved by the Institutional Animal Care and Use Committee at BIDMC and all animals were treated in compliance with the "Guide for the Care and use of Laboratory Animals" (8th edition, National Institutes of Health, 2010). Both male and female adult Wistar rats (Charles River, Indianapolis, IN, USA) were used at an ideal weight of 400-450g and 275-300g respectively (n=7 per group). Following a 48-hour acclimation period, rats were anesthetized with Isoflurane (2.5-3%) via an induction chamber and sustained by conical mask. Respiratory rate and anesthesia were monitored throughout the entire procedure. With the animal in dorsal decumbency, one of the common carotid arteries (CCA) was dissected and exposed from the sternal notch to the bifurcation through a midline neck incision. Once arterial control was obtained, a 2F Fogarty catheter was introduced through an arteriotomy in the external carotid artery. To ensure complete circumferential endothelial denudation the balloon was advanced, inflated and withdrawn towards the entry point with constant rotation. This procedure was repeated for a total of three passages. Subsequently, the balloon-injured region of the CCA was infused intraluminally with either saline, PEI only, non-targeting control siRNA with PEI (PEI+control siRNA; 50μM in 50-75μL), or TSP-2 siRNA with PEI (PEI+TSP-2 siRNA; 25μM in 50-75μL). For adequate transfection, arterial segments were isolated with vascular clamps and distended for 15 minutes. Transfection time of 15 minutes was selected, taken into consideration the time and technical constraints within the operating room. Afterwards the CCA was flushed with saline. Once hemostasis was confirmed the wound was approximated with a subcuticular running suture (5-0 Vicryl Plus). Meloxicam was administered as postoperative analgesia with an additional dose 24 hours later (1 mg/kg intraperitoneal).

Tissue harvest

At 24 hours and 21 days following the initial operative procedure, rats were euthanized. Denuded CCA along with contralateral non-operated control CCA, which will be referred to as non-denuded CCA, were harvested. While under deep anesthesia, a midline thoracotomy was made for cardiac saline perfusion to clear arterial vessels of blood. The denuded and the non-denuded CCA were then fixed in formalin 10% at 4°C for 24 hours. Before embedding in paraffin, each CCA was cut at the midpoint and, as a result, analysis started at the mid-segment of the balloon-injured arteries.

Quantitative real-time-polymerase chain reaction (qRT-PCR)

RNA was isolated and extracted with the Recoverall™ Total Nucleic Acid Isolation Kit (Life Technologies, Grand Island, NY, USA). Upon purification, samples were quantified using a NanoDrop spectrophotometer. Equal amounts of total RNA from the different samples were subjected to standard qRT-PCR method. Rat specific primers for TSP-2 and the housekeeping gene β_2-microglobulin were obtained from Integrated DNA technologies (Coralville, IA, USA). TSP-2 mRNA levels were normalized to β_2-microglobulin levels and all gene amplifications reactions were carried out in duplicate. The $2^{-\Delta\Delta CT}$ method was used to calculate relative TSP-2 mRNA expression levels (relative to non-denuded contralateral CCA). Information about the reagents and TSP-2 primer sequences is provided in Supplementary Table 1.

Histology

Paraffin-embedded cross sections of 6µm were obtained for histology and immunohistochemical analysis. IH was determined with the Verhoef-van Giesson stain by measuring the intima/media area ratio. To provide a representative sample, six equally separated cross sections were analyzed per CCA to average along the length of the injured artery. Collagen deposition was assessed with a Masson's Trichrome stain and presented as a percentage of the total area. Information about the reagents is provided in Supplementary Table 1.

Immunohistochemical analysis

Protein expression: Standard fluorescence immunohistochemistry protocol was followed to evaluate protein expression of TSP-2, transforming growth factor-βeta (TGF-β) and matrix metalloproteinase-9 (MMP-9). Information about specific antibodies and reagents is provided in Supplementary Table 1. To quantify protein expression we used the following arbitrary scoring system. Density of expression scoring: 1 = minimal, 2 = mild, 3 = moderate, 4 = strong, and 5 = extensive. Dissemination of expression scoring: 1 = minimal expression in intima, media or adventitia, 2 = limited expression in one cell layer, 3 = limited expression in two cell layers, 4 = expression in all cell layers, and 5 = extensive expression throughout the specimen. TSP-2 protein expression was quantified using both density and dissemination scoring system. TGF-β protein expression was quantified using only density scoring system. Positive staining of MMP-9 was predominantly in the intima, and hence only density scoring system was applied. Data for TSP-2 and TGF-β are presented as arbitrary scores, while data for MMP-9 are presented as a percentage of total intima area. Cellular proliferation: Proliferative cells were detected with a commercial Ki67 assay. Standard immunohistochemical staining protocol was performed with DAB substrate buffer and counterstained with hematoxylin, after which sections were mounted. Data are presented as number of positive cells per mm^2. Information about the reagents is provided in Supplementary Table 1.

Macrophage infiltration: Infiltration was assessed with anti-CD68, a pan-macrophage marker. Polarization of macrophages was determined by co-staining of DAPI, anti-CD68, and anti-iNOS for detection of M1-macrophages and DAPI, anti-CD68, and anti-CD206 for M2-macrophages on two separate cross-sections. Standard fluorescence immunohistochemistry protocol was followed and information about the antibodies is provided in Supplementary Table 1. Quantification was performed by counting the number of positive cells per mm^2 of the specimen. For all immunohistochemical stains two equally separated cross-sections were analyzed per specimen. Analysis was conducted by two observers in a blinded fashion. ImageJ (v1.49) was used as analysis software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Differences between non-denuded and denuded CCA were analyzed using the paired sample t-test, since these were tested within the same animal. To assess the effect of treatment in the denuded arteries, One-way analysis of variance (ANOVA) was performed for multiple comparisons followed by Dunnett's post hoc test, with PEI+TSP-2 siRNA as the category group. Normally distributed continuous variables are presented as mean ± S.E.M. All tests were two-sided and data was considered statistically significant when p-value <0.05. Statistical analysis was performed using IBM SPSS Statistics 23 (IBM Corp, Armonk, NY, USA) .

RESULTS

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on TSP-2 gene expression

TSP-2 gene expression was compared between the denuded saline treated CCA and contralateral non-denuded CCA by qRT-PCR at 24 hours and 21 days. To further investigate whether intraluminal delivery of siRNA alters gene expression of TSP-2 *in vivo*, CCA were analyzed at 24 hours and 21 days following denudation and siRNA transfection. PCR data are normalized to non-denuded CCA and subsequently expressed as a fold change.

Twenty-four hours after denudation, TSP-2 gene expression was significantly upregulated in denuded CCA (2.40±0.42) compared to non-denuded CCA (1.00±0.04, P=0.006; Figure 1 A). Twenty-one days after denudation, the upregulation of TSP-2 mRNA was maintained in the denuded CCA (3.73±0.95) compared to non-denuded controls (1.04±0.13, P=0.028; Figure 1 B).

Twenty-four hours after denudation and transfection, PEI+TSP-2 siRNA treatment significantly reduced gene expression of TSP-2 (0.77±0.09) compared to saline (2.40±0.42, P=0.045). There were no significant differences between PEI+TSP-2 siRNA and PEI only (1.60±0.71, P=0.461) or PEI+control siRNA (1.76±0.47, P=0.294) treatment (Figure 1 A). Twenty-one days after denudation and siRNA transfection, PEI+TSP-2 siRNA treatment

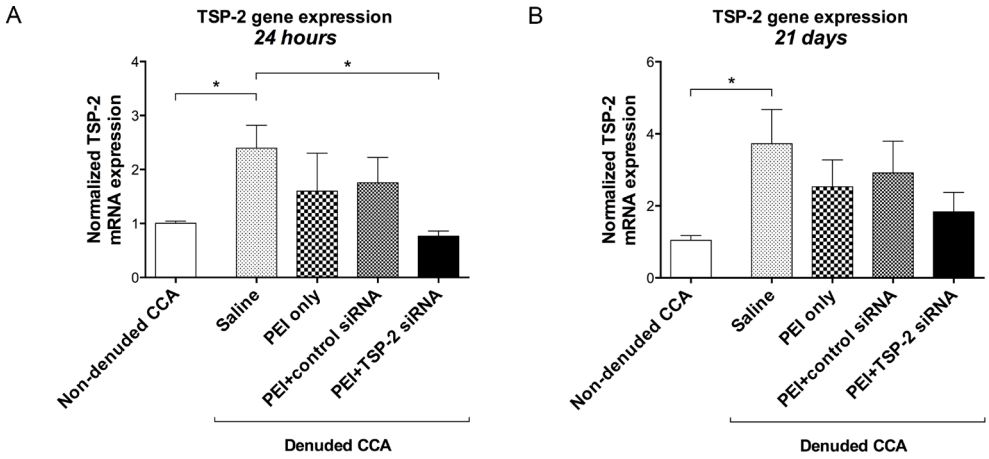

Figure 1. Relative gene expression of TSP-2: Fold change in TSP-2 mRNA expression in all groups as relative to non-denuded contralateral CCA at 24 hours (**A**) and 21 days (**B**). Data are presented as mean ± S.E.M, * P<0.05. N=5.

only showed a trend towards reduced gene expression (1.83±0.54) with no significant differences compared to saline (3.73±0.95, P=0.206), PEI only (2.53±0.75, P=0.880), or PEI+control siRNA (2.92±0.88, P=0.682) treatment (Figure 1 B).

These data suggest that, TSP-2 gene expression was significantly upregulated following endothelial denudation in the rat carotid balloon injury model, thus validating the model. While gene silencing was not sustained over a twenty-one-day period, these data suggest that a single 15-minute localized intraluminal infusion of target-specific siRNA is effective at achieving short-term gene silencing.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on TSP-2 protein expression

TSP-2 protein expression was compared between the denuded saline treated CCA and the contralateral non-denuded CCA by immunohistochemistry at 21 days. To further investigate whether intraluminal delivery of siRNA alters TSP-2 protein expression, CCA were analyzed at 21 days following denudation and siRNA transfection. Quantification of TSP-2 protein expression was based on density and dissemination of TSP-2 expression in CCA. Data are expressed as arbitrary scores.

Twenty-one days after denudation, TSP-2 protein density was increased in denuded CCA (3.47±0.64) compared to non-denuded CCA (1.25±0.10, P=0.040; Figure 2 A). Additionally, dissemination of TSP-2 protein was higher in denuded CCA (2.88±0.39) compared to non-denuded CCA (1.44±0.06, P=0.043; Figure 2 B).

Twenty-one days after denudation and siRNA transfection, PEI+TSP-2 siRNA treatment significantly reduced the density of TSP-2 protein expression (1.20±0.11) compared to saline (3.47±0.64, P=0.007), PEI only (3.22±0.27, P=0.022), or PEI+control siRNA (3.09±0.41, P=0.020) treatment (Figure 2 A). Similarly, transfection with PEI+TSP-2 siRNA significantly reduced the dissemination of TSP-2 protein expression (1.86±0.25) compared to PEI+control siRNA (3.27±0.14, P=0.019), whereas near significant differences were observed with saline (2.88±0.39, P=0.094) or PEI only (3.04±0.46, P=0.069) treatment (Figure 2 B). Representative images of TSP-2 protein expression are depicted in Figure 2 C.

These data suggest that, TSP-2 protein expression was significantly upregulated up to 21 days following endothelial denudation in the rat carotid balloon injury model, thus validating the model. Although significant gene silencing of TSP-2 was not sustained by PEI+TSP-2 siRNA treatment over a 21-day period, TSP-2 protein knockdown was achieved after a single intraluminal transfection with TSP-2 siRNA.

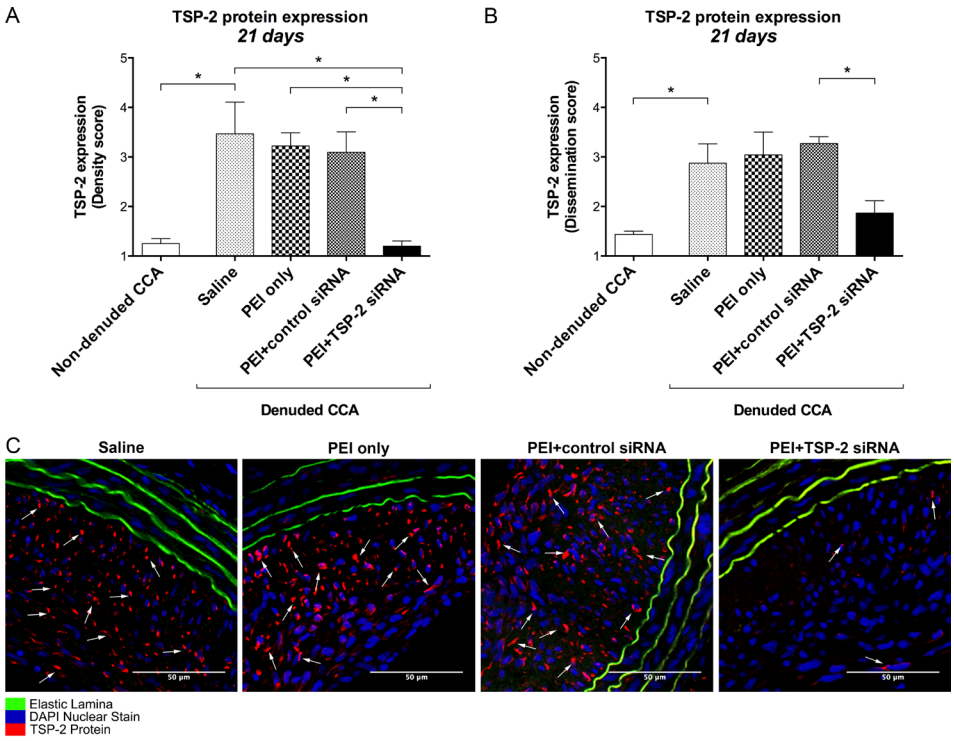

Figure 2. TSP-2 protein expression: Quantification of TSP-2 protein expression in all groups at 21 days based on density (**A**) and dissemination scores (**B**). Data are expressed as arbitrary scores (1-5) and are presented as mean ± S.E.M, * P<0.05. N=4. (**C**) Representative images at 21 days of all denuded CCA stained for TSP-2 protein (red), nuclei (blue), and elastic lamina (green autofluorescence). Magnification - 400x, scale bar - 50µm.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on cell proliferation

Cellular proliferation is one of the key events in the development of IH.[3] Immunostaining for Ki-67, a marker of cellular proliferation, was performed in all CCA at 21 days. Data are expressed as number of proliferating cells/mm^2. Twenty-one days after denudation, cell proliferation was significantly increased in the denuded saline CCA (33.8±5.0) compared to non-denuded CCA (13.4±1.4, P=0.017; Figure 3 A).

Twenty-one days after denudation and transfection, PEI+TSP-2 siRNA treatment significantly decreased proliferation (14.4±2.5) compared to saline (33.8±5.0, P=0.048) or PEI only (45.4±5.5, p=0.003) treatment. There was no significant difference observed between PEI+TSP-2 siRNA and PEI+control siRNA (30.1±6.4, P=0.119) treatment (Figure 3 A). Representative images of cellular proliferation are depicted in Figure 3 B.

These data suggest that, arterial denudation increased cell proliferation and this increase in proliferation could be curtailed by single intraluminal transfection with TSP-2 siRNA.

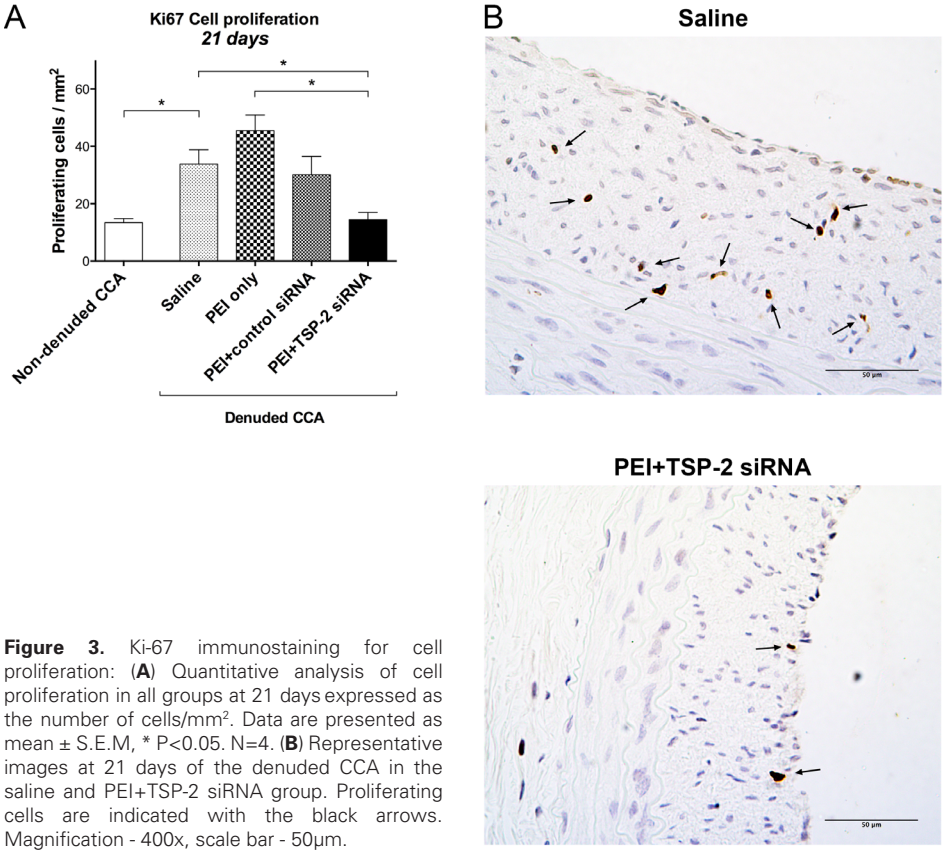

Figure 3. Ki-67 immunostaining for cell proliferation: (**A**) Quantitative analysis of cell proliferation in all groups at 21 days expressed as the number of cells/mm^2. Data are presented as mean ± S.E.M, * P<0.05. N=4. (**B**) Representative images at 21 days of the denuded CCA in the saline and PEI+TSP-2 siRNA group. Proliferating cells are indicated with the black arrows. Magnification - 400x, scale bar - 50µm.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on collagen deposition

The final lesion of IH is made up of collagen.[14] Hence, collagen deposition was measured using Masson's Trichrome stain at 21 days. Data are presented as a percentage stained/mm^2. Twenty-one days after denudation, collagen deposition was significantly increased in the denuded saline CCA (47.0±2.0%) compared to the non-denuded CCA (10.2±1.2%, P<.001; Figure 4 A).

Twenty-one days after denudation and transfection, PEI+TSP-2 siRNA treatment significantly reduced collagen deposition (32.2±0.5%) compared to saline (47.0±2.0%, P=0.001) or PEI+control siRNA (40.9±3.6%, P=0.041) treatment, whereas a near significant difference was observed with PEI only (40.4±1.5%, P=0.056) treatment (Figure 4 A). Representative images of collagen deposition are depicted in Figure 4 B.

These data suggest that, arterial denudation increased collagen deposition and this increase in collagen could be mitigated by single intraluminal transfection with TSP-2 siRNA.

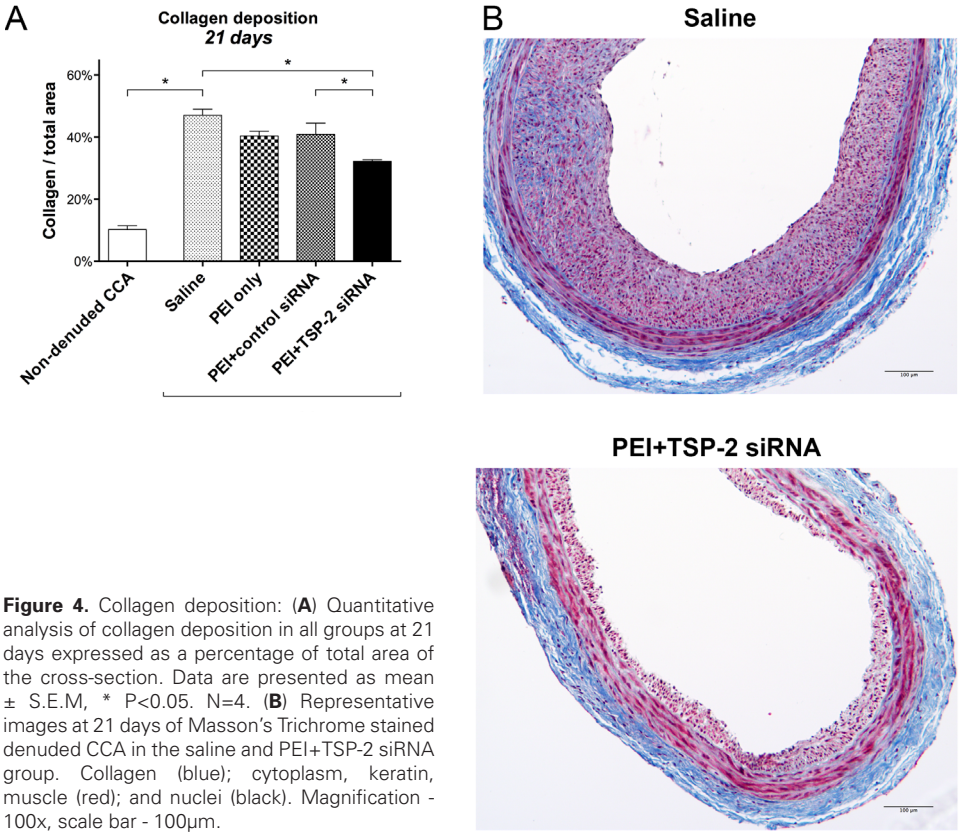

Figure 4. Collagen deposition: (**A**) Quantitative analysis of collagen deposition in all groups at 21 days expressed as a percentage of total area of the cross-section. Data are presented as mean ± S.E.M, * P<0.05. N=4. (**B**) Representative images at 21 days of Masson's Trichrome stained denuded CCA in the saline and PEI+TSP-2 siRNA group. Collagen (blue); cytoplasm, keratin, muscle (red); and nuclei (black). Magnification - 100x, scale bar - 100µm.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on macrophage infiltration

To identify early and long-term inflammatory events, we examined CCA for infiltration of macrophages at 24 hours and 21 days after transfection by immunostaining for CD68 antigen. Data are expressed as CD68+ cells/mm^2.

Denudation triggered macrophage activation in denuded saline CCA both at 24 hours (263.5±50.3) and 21 days (223.4±62.6) compared to their contralateral non-denuded CCA (16.8±3.7, P=0.004 and 12.5±3.1, P=0.022 respectively; Figure 5 A & 5 B).

Twenty-four hours after denudation and transfection, extensive macrophage infiltration was observed in all treatment groups. PEI+TSP-2 siRNA (394.7±63.6) treatment showed no significant differences as compared to saline (263.5±50.3, P=0.316), PEI only (402.9±63.1, P=0.999) or PEI+control siRNA (378.9±77.6, P=0.996) treatment (Figure 5 A).

Twenty-one days after denudation and transfection, the increase in macrophage infiltration that was observed at 24 hours with PEI only, PEI+control siRNA or PEI+TSP-2 siRNA treatment was significantly diminished. Similar to 24 hours, after 21 days, macrophage infiltration with PEI+TSP-2 siRNA (232.0±39.8) treatment was not significantly different compared to saline (223.4±62.6, P=0.998), PEI only (227.0±15.8, P>0.99), or PEI+control siRNA (275.3±39.9, P=0.887) treatment (Figure 5 B).

These data suggest that, arterial denudation increased macrophage infiltration and this increase in macrophage infiltration could not be suppressed by single intraluminal transfection with TSP-2 siRNA.

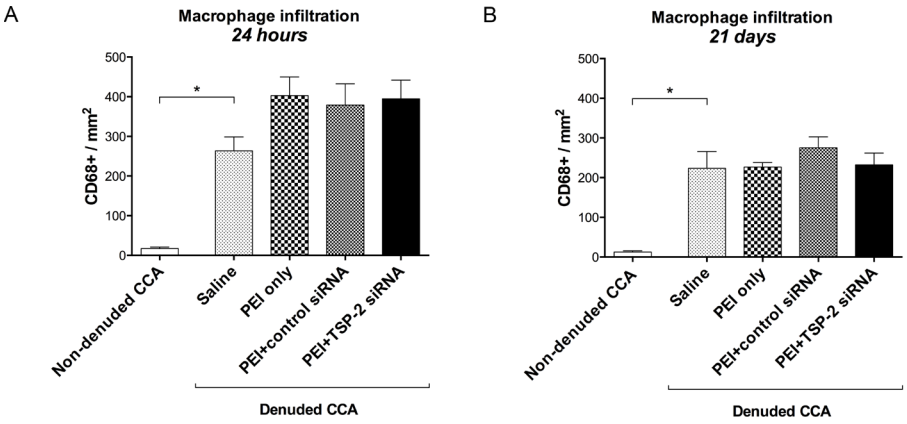

Figure 5. Total macrophage infiltration: Quantification of total macrophage infiltration in all groups expressed as CD68+ cells at 24 hours (**A**) and 21 days (**B**). Data are presented as mean ± S.E.M., * P<0.05. N=4-6.

Effect of TSP-2 siRNA transfection on macrophage polarization in denuded CCA

To further evaluate the phenotype of the infiltrating macrophages, we measured M1 and M2 macrophage polarization in the denuded CCA at 24 hours and 21 days after denudation and transfection. Quantification of polarization was performed by calculating the M1/M2 ratio, which represents macrophage heterogeneity. Any value greater than one correlates with a pro-inflammatory phenotype and a value less than one with an anti-inflammatory phenotype of scavenging and remodeling. Data are expressed as a ratio.

Twenty-four hours after denudation and transfection, all treatment groups demonstrated a pro-inflammatory phenotype with M1/M2 ratios above one. Moreover, PEI+TSP-2 siRNA (1.19±0.17) treatment was not significantly different from saline (1.39±0.19, P=0.880), PEI only (1.20±0.17, P>0.99), or PEI+control siRNA (1.31±0.35, P=0.970) treatment (Figure 6 A).

Twenty-one days after denudation and transfection, PEI+TSP-2 siRNA treatment had a significantly lower M1/M2 ratio (0.88±0.04) compared to saline (1.48±0.14, P=0.049), whereas no significant differences were observed with PEI only (1.03±0.21, P=0.842) or PEI+control siRNA (1.12±0.18, P=0.590) treatment (Figure 6 B). Representative images of M1 and M2 macrophage infiltration are depicted in Figure 6 C & 6 D.

These data suggest that at 24 hours a single intraluminal transfection with TSP-2 siRNA could not improve the M1/M2 ratio. However, by 21 days, it could significantly reduce the M1/M2 ratio. Interestingly, although the total macrophage counts were similar between saline and PEI+TSP-2 siRNA treatment, the latter treatment group demonstrated increased anti-inflammatory M2 macrophages.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on regulation of TGF-β and MMP-9

Possible downstream targets of TSP-2, TGF-β and MMP-9, which are known to play a role in vascular injury, were investigated. We analyzed TGF-β protein expression at 21 days using immunohistochemistry. Quantification of TGF-β protein expression was based on density of protein stained in CCA. Data are expressed as arbitrary scores.

Twenty-one days after denudation, TGF-β protein expression was significantly increased in denuded saline CCA (3.11±0.22) compared to non-denuded CCA (1.06±0.04, P=0.003; Figure 7 A). Twenty-one days after denudation and siRNA transfection, PEI+TSP-2 siRNA treatment significantly further increased TGF-β expression (4.47±0.18) compared to saline (3.11±0.22, P=0.012) treatment. No significant differences were found compared to PEI only (3.90±0.21, P=0.340) and PEI+control siRNA (3.58±0.35, P=0.097) treatment (Figure 7 A). Representative images of TGF-β protein expression are depicted in Figure 7 B.

We analyzed MMP-9 protein expression at 21 days using immunohistochemistry. Data are presented as a percentage stained/mm^2 of the intima, since positive staining was solely observed in the intima of CCA.

Twenty-one days after denudation, MMP-9 expression was significantly increased in the denuded saline CCA (64.9±2.9%) as compared to non-denuded CCA (7.1±1.4%, P<.001; Figure 7 C).

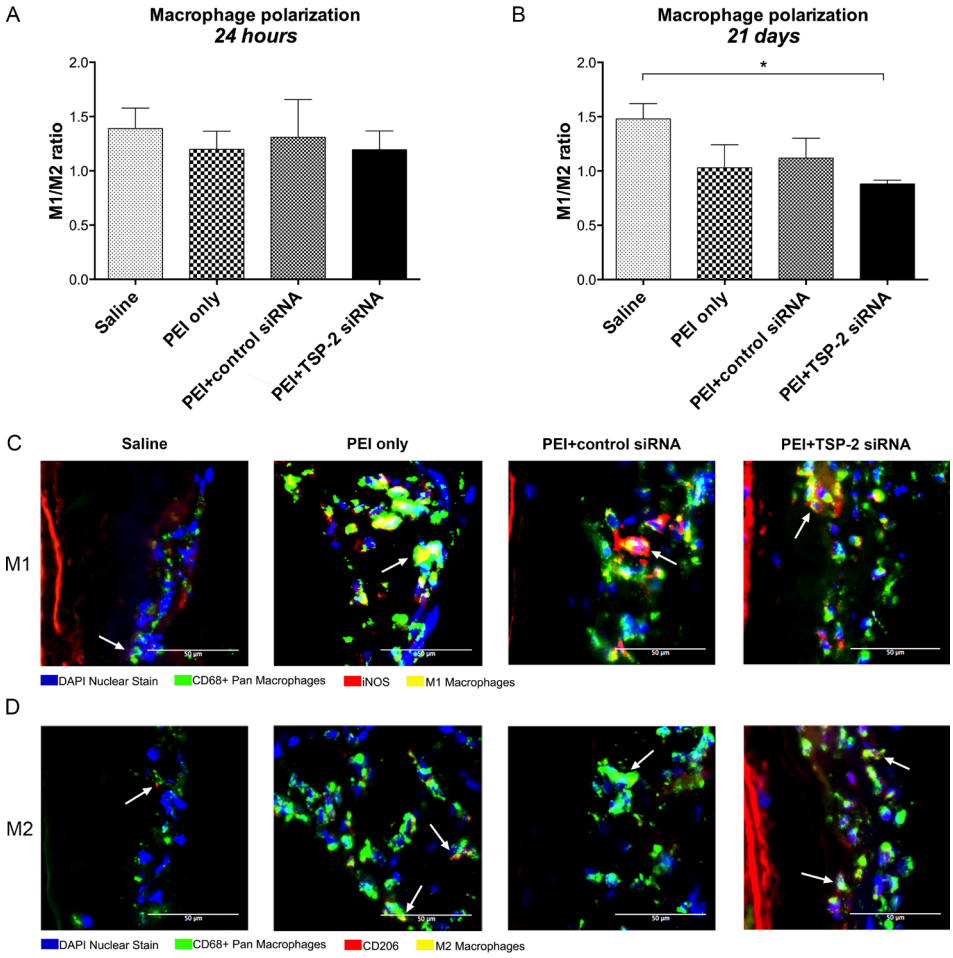

Figure 6. Macrophage polarization: Quantification of macrophage polarization in all denuded CCA expressed as M1/M2 ratio at 24 hours (**A**) and 21 days (**B**). Data are presented as mean ± S.E.M., * P<0.05. N=4. Representative images at 21 days of denuded CCA in all groups depicting M1 and M2 macrophage polarization. Pro-inflammatory M1 macrophages were co-stained with DAPI (blue), CD68 (green), and iNOS (red) (**C**), whereas anti-inflammatory pro-tissue repair M2 macrophages were co-stained with DAPI (blue), CD68 (green), and CD206 (red) (**D**). White arrows indicate these macrophages respectively. Magnification - 400x, scale bar - 50µm.

Twenty-one days after denudation and transfection, PEI+TSP-2 siRNA treatment significantly reduced MMP-9 expression (37.6±2.6%) compared to saline (64.9±2.9%, P<.001), PEI only (57.6±1.4%, P<.001), and PEI+control siRNA (56.0±2.8%, P=0.001) treatment (Figure 7 C). Representative images of MMP-9 protein expression are depicted in Figure 7 D. These data suggest that, arterial denudation increased TGF-β and MMP-9 protein expression up to 21 days. A single intraluminal transfection with TSP-2 siRNA further increased TGF-β

expression while reducing MMP-9 expression indicating that TSP-2 could play a role in the modulation of these two proteins in an arterial injury model.

Effect of arterial denudation and subsequent TSP-2 siRNA transfection on intimal hyperplasia

Finally, we evaluated the effect of TSP-2 silencing on IH by measuring intima/media area ratios using Verhoef-van Giesson stain at 21 days. Twenty-one days after denudation, intima/media area ratio was significantly increased in the denuded saline CCA (1.29±0.16) compared to non-denuded CCA (0.049±0.00, P=0.001; Figure 8 A)

Twenty-one days after denudation and transfection, PEI+TSP-2 siRNA treatment reduced intima/media area ratio (0.97±0.19) compared to saline (1.29±0.16, P=0.480), PEI only (1.49±0.16, P=0.147) or PEI+control siRNA (1.24±0.23, P=0.605) treatment. However, none of these differences were statistically significant (Figure 8 A). Representative images of intima/media ratios are depicted in Figure 8 B. These data suggest that, arterial denudation led to IH development by 21 days. However, a single intraluminal transfection with TSP-2 siRNA was not sufficient to significantly suppress IH development.

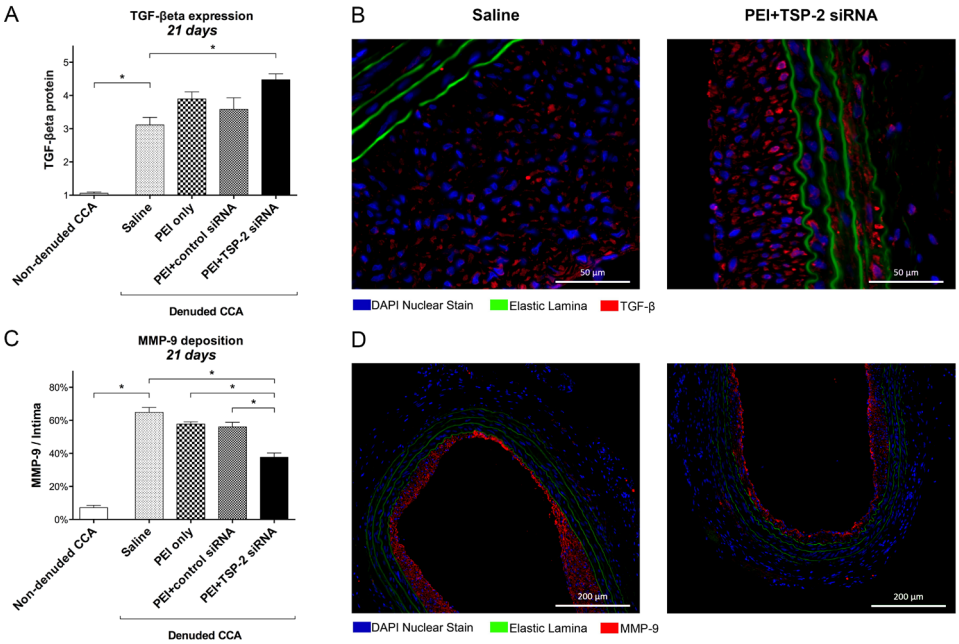

Figure 7. Downstream targets: (**A**) Quantification of TGF-β protein expression in all groups at 21 days based on arbitrary density scores (1-5). (**B**) Representative images at 21 days of the denuded CCA in the saline and PEI+TSP-2 siRNA group stained for TGF-β (red), nuclei (blue), and elastic lamina (green autofluorescence). Magnification - 400x, scale bar - 50µm. (**C**) Quantification of MMP-9 expression in all groups 21 days expressed as a percentage of the intima. (**D**) Representative images at 21 days of the denuded CCA in the saline and PEI+TSP-2 siRNA group stained for MMP-9 (red), nuclei (blue), and elastic lamina (green autofluorescence). Magnification - 100x, scale bar - 200µm. Data are presented as mean ± S.E.M, * P<0.05. N=4.

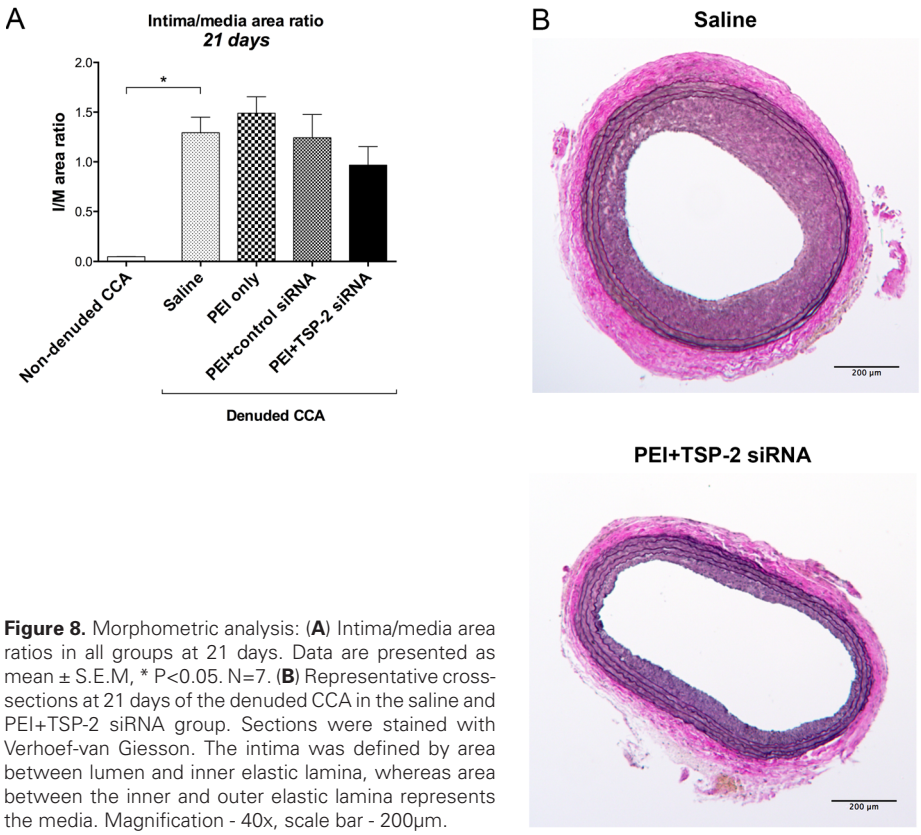

Figure 8. Morphometric analysis: (**A**) Intima/media area ratios in all groups at 21 days. Data are presented as mean ± S.E.M, * P<0.05. N=7. (**B**) Representative cross-sections at 21 days of the denuded CCA in the saline and PEI+TSP-2 siRNA group. Sections were stained with Verhoef-van Giesson. The intima was defined by area between lumen and inner elastic lamina, whereas area between the inner and outer elastic lamina represents the media. Magnification - 40x, scale bar - 200µm.

DISCUSSION

ECM proteins are recognized as important modulators that contribute to arterial restenosis and graft failure, impairing remodeling and matrix stability.[15] TSP-2 is an ECM protein, primarily produced by smooth muscle cells and fibroblasts, and recognized as an ECM modulator following injury. Recent studies have associated TSP-2 with tissue repair, collagen synthesis, bone growth, and angiogenesis. In 1999, Kyriakides et al reported deposition of discontinuous collagen fibrils, highly vascularized granulation tissue, and enhanced cellularity in wounds lacking TSP-2.[12] As a result, these wounds demonstrated an accelerated rate of healing compared to wild-type mice. In addition, TSP-2 null mice were associated with induced endothelial cell migration, leading to enhanced neovascularization, as well as impaired platelet aggregation, which would be important in prevention of thromboembolic events.[16]

The present study expands on our previous *in vitro* and *ex vivo* findings demonstrating efficient transfection of endothelial and smooth muscle cells with siRNA that resulted in consistent and reproducible silencing of target genes.[17,18] Our current study provides further evidence of effective gene silencing with siRNA in an animal model. We demonstrated that localized intraluminal gene knockdown of TSP-2 by siRNA, possibly through TGF-β and MMP-9-dependent pathways, resulted in significant inhibition of protein expression, cell proliferation, and collagen deposition, persisting up to 21 days following transfection. Furthermore, TSP-2 knockdown induces anti-inflammatory repair and remodeling in a balloon-injured arterial wall.

Numerous studies have demonstrated efficacious silencing of genes in IH animal models through perivascular application or systemic administration of siRNA, while only a limited number of studies were successful with localized intraluminal transfection.[19-22] Of these studies, prolonged transfection times were applied, whereas other reports used viral vectors for more adequate transfection. In contrast, these results demonstrate, for the first time, that both gene and protein expression of TSP-2 are significantly inhibited after localized intraluminal transfection of only 15 minutes with a non-viral delivery system. Although there were no differences in gene expression at 21 days, a prolonged inhibition of TSP-2 protein expression was demonstrated in PEI+TSP-2 siRNA-treated group. These results clearly indicate effective delivery of siRNA to the arterial wall under clinically applicable conditions. Moreover, localized intraluminal transfection poses a viable alternative to avoid systemic effects, which are known concerns of sustained release perivascular and systemic delivery. Administration of high doses to achieve sufficient transfection and exposure of non-target tissues could affect siRNA specificity and induce unintended systemic effects.[23] The findings of this study are relevant to gene therapy safety and the feasibility of clinical application within the time and technical constraints of the operating room.

Collagen deposition at 21 days was significantly reduced in the arterial wall following TSP-2 silencing. These results are consistent with previous reports of accelerated wound healing and support a disordered organization of collagenous matrix in the absence of TSP-2. Specifically, it has been shown that TSP-2 interacts with several ECM proteins, including collagen, fibrinogen, and fibronectin. Structural matrix abnormalities and the complexity in which TSP-2 modulates the ECM are partially elucidated in dermal fibroblasts. In TSP-2 null mice, tissue-transglutaminase (tTG) and matrix metalloproteinase-2 (MMP-2) were implicated in degrading many matrix proteins, including collagen. Its subsequent inhibitors reversed proteolysis and increased cross-linking of the ECM.[24] Our data supports these findings and suggests modulating capabilities of TSP-2 on downstream regulating ECM proteins, in particular TSP-2-mediated reduction of MMP-9, which may maintain vessel circumference. Additionally, convincing evidence exists that MMP-9 contributes to arterial lesion growth and impaired wound healing by regulating smooth muscle cell migration and replication.[25-27] Therefore, lower MMP-9 levels might alter the severity of IH. The regulation of MMP-9 by TSP-2 has not been extensively studied; however, Krady et al. demonstrated

that TSP-2 seems to regulate extracellular levels of MMP-9 in a spatiotemporal fashion in response to ischemic injury in a mouse hindlimb model.[28] Although we found reduced MMP-9 protein levels after TSP-2 knockdown, others have reported increased levels of MMP-2 and MMP-9 in TSP-2 null mice.[28,29] The reason for this discrepancy is unclear, but emphasizes the fragile equipoise of catabolic degradation and upregulation of MMPs through their subsequent tissue inhibitors.

In contrast to the present results showing significant inhibition of cell proliferation following TSP-2 knockdown in an animal model, our previous *in vitro* study could not demonstrate a similar correlation for smooth muscle cells.[9] Through Rac-regulated modulation, TSP-2 synthesis has been shown to inhibit cell proliferation of endothelial cells, yet it is hypothesized that thrombospondins stimulate cell growth in smooth muscle cells.[30,31] Recently, using human aortic vascular smooth muscle cells, TSP-2 was associated with 3.1-fold rise in chemotaxis compared to serum-free media that resulted in a 50% increase of proliferation *in vitro*.[32] These results further support the idea of proliferative cell inhibition by TSP-2 siRNA that may potentially attenuate IH formation.

Furthermore, we present evidence that siRNA-mediated knockdown of TSP-2 affects macrophage infiltration and polarization in the latter stages of IH. In PEI+TSP-2 siRNA-treated group, we observed a M2 polarization at 21 days that is associated with an anti-inflammatory phenotype. The spectrum of functional phenotypes of macrophages is induced by various cytokines, of which IL-4, but also TGF-β plays a key role.[33] While TSP-1, a different isoform of TSP, has been shown to activate latent TGF-β that functions as an anti-inflammatory cytokine, it is suggested that TSP-2 competitively inhibits activation of TGF-β.[34] The current study provides evidence that TSP-2 silencing leads to higher levels of TGF-β. It is likely that this increase in TGF-β may promote the anti-inflammatory M2 macrophage phenotype known to induce repair and remodeling. Seemingly contradictory findings are reported regarding the role of TGF-β in restenosis. While some studies found that overexpression of TGF-β after arterial injury decreased neointima formation, vast majority suggest that TGF-β increases smooth muscle cell proliferation and migration.[35] It is important to note that this paradox is also reported in malignancies (tumor suppressor vs. promoter) and our study adds to the growing body of literature on the temporal and context-dependent function of TGF-β. Nevertheless, TSP-2 modulates TGF-β downstream most likely through TSP-1-mediated activation, which could be critical for macrophage-vessel interactions. Further studies will be needed to understand the role of TSP-2 dependent TGF-β induction in arterial injury.

siRNAs are extremely hydrophilic and are quickly degraded in serum, contributing to their short half-lives *in vivo*. Various chemical modifications, transfection agents and delivery methods have been evaluated to increase stability and transfection. In the present study we used PEI as a transfection agent to enhance entry of siRNA into the cell and achieve increased silencing of the target gene. It is interesting to note that our results indicate enhanced cell proliferation and increased IH for the PEI only group. Though PEI combined with target siRNA exerts protective vascular effects, PEI only may induce specific alterations

in the transcriptome and increase expression of off-target genes.[36] Our research group recently identified 213 differentially expressed genes that are modulated due to transfection with PEI only. Major genes linked to vascular dysregulation primarily include inflammation-related and cell proliferative alterations (e.g. IL1A, STAT-4, CCL8, SFN, and CSF2). (37) These data are supported by our current findings of increased CD68+ macrophage infiltration at 24 hours of all PEI combined treatment groups compared to saline. These off-target effects of PEI are significant and warrant the development of efficacious yet non-toxic siRNA delivery agents.

Contrary to expectations, this study did not find a significant reduction of IH after TSP-2 knockdown. This outcome could be attributed to the complex pathogenesis of IH involving crosstalk among various cellular and molecular pathways or to the smaller animal sample size. While single gene knockout highlights the potential of siRNA, simultaneous silencing of multiple genes represents a plausible method to effectively attack IH. Our research group has already demonstrated multiple gene silencing *in vitro* and will undertake further studies to investigate this hypothesis *in vivo*.[37]

This study has limitations that should be addressed. First, we used a rat carotid balloon angioplasty injury model to mimic diseased vessels after surgical manipulation. To better comprehend the processes leading to atherosclerosis and IH, an animal model of graft failure would be preferred. However, the rat carotid balloon injury model serves as a valuable proof-of-concept study to verify *in vitro* results. Furthermore, in addition to the current study demonstrating promising results extending to 21 days, further research is needed to investigate the long-term effects of TSP-2 silencing on vascular restenosis.

In conclusion, we demonstrated that siRNA-mediated knockdown of TSP-2 gene expression after vascular injury in an *in vivo* animal model effectively modulates TGF-β and MMP-9 that are known to contribute to IH. Effective target-specific gene silencing with shortened localized intraluminal transfection times is feasible under clinically applicable conditions. This work justifies further research of local siRNA treatment for IH and vascular restenosis.

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SUPPLEMENTARY

Supplementary Table 1: List of Reagents

siRNA		
Reagent	**Catalog Number**	**Company**
Silencer® Select Thbs-2 siRNA	s146414	Life Technologies, Grand Island, NY, USA
Silencer® Select Negative Control #1 siRNA	4390844	Life Technologies, Grand Island, NY, USA
qRT-PCR		
Reagent	**Catalog Number**	**Company**
IScript cDNA Synthesis Kit	1708891	Bio Rad, Hercules, CA, USA
Brilliant III SYBR® master mix with ROX	600882	Agilent Technologies, Santa Clara, CA, USA
Primers (Integrated DNA Technologies, Coralville, IA, USA)		
Gene	Forward 5'- 3'	Reverse 5' - 3'
TSP-2	GGACAACTGCAGGCTTGTGTTCAA	AGTCTGTCTCGGTGATGGCATTGT
β_2-microglobulin	ACACTGAATTCACACCCACCGAGA	TGATTACATGTCTCGGTCCCAGGT
Histology		
Reagent	**Catalog Number**	**Company**
Verhoef-van Giesson stain	NC9239432	Fisher Scientific, Pittsburgh, PA, USA
Masson's Trichrome stain	KTMRT	American MasterTech Scientific, Lodi, CA, USA
Protein Expression		
Protein	**Primary Antibody Catalog Number, Company**	**Secondary Antibody, Catalog Number, Company**
TSP-2	ab84469, Abcam, Cambridge, MA, USA	Cy5 ab6719, Abcam, Cambridge, MA, USA
TGF-β	ab66043, Abcam, Cambridge, MA, USA	Cy5 ab6719, Abcam, Cambridge, MA, USA
MMP-9	ab38988, Abcam, Cambridge, MA, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA
Cell Proliferation		
Reagent	**Catalog Number**	**Company**
Ki67	ab1667	Abcam, Cambridge, MA, USA
Macrophage Infiltration		
Antibody	**Primary Antibody Catalog Number, Company**	**Secondary Antibody, Catalog Number, Company**
CD-68 (pan macrophage marker)	MCA341GA, AbD Serotec, Raleigh, NC, USA	Alexa Fluor 488 715-545-150, Jackson ImmunoResearch, West Grove, PA, USA
iNOS (M1 marker)	ab136918, Abcam, Cambridge, MA, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA
CD206 (M2 marker)	MCA2155T, AbD Serotec, Raleigh, NC, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA

CHAPTER 10

SUCCESSFUL DUAL GENE KNOCKDOWN IN AN ARTERIAL INJURY MODEL OF VASCULAR REMODELING

In preparation to submit

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ABSTRACT

Rationale

Overexpression of thrombospondin-2 (TSP-2) and myristoylated alanine-rich C kinase substrate (MARCKS) occurs in the development of proliferative vascular lesions such as restenosis following vascular interventions.

Objective

Here we investigate the effects of single or dual gene knockdown with small interfering RNA (siRNA) on cell proliferation, collagen deposition, and the inflammatory response in an *in vivo* model.

Methods and Results

Following arterial injury in a rat carotid balloon angioplasty model the artery was treated with dual siRNA (siTSP-2+siMARCKS), single siRNA (siTSP-2 or siMARCKS), or saline. Arterial segments were analyzed at 24 hours or 21 days using qRT-PCR and immunohistochemistry. TSP-2 and MARCKS gene and protein expression were upregulated early after arterial injury. Compared to saline treated artery, specific silencing was observed after simultaneous knockdown of TSP-2 and MARCKS, which was more pronounced than single gene knockdown. Dual gene knockdown led to the lowest rate of proliferating cells after 21 days, while collagen deposition was reduced but similar to the levels of single gene knockdown. This was reflected by the lower expression of matrix metalloproteinase-9 (MMP-9) compared to saline treatment. Dual siRNA and single siMARCKS treatment led to reduced macrophage infiltration compared to saline or single siTSP-2 treatment, which is likely related to differences in transforming growth factor-βeta (TGF-β). Moreover, single and dual siRNA treatment groups induced anti-inflammatory M2 macrophages at 21 days compared to treatment with saline. No significant differences were found after examining intima/media area ratios.

Conclusions

Dual gene silencing of TSP-2 and MARCKS results in sufficient gene and protein knockdown, reduced cell proliferation and collagen deposition, and induced inflammatory repair and remodeling. Thus, targeting multiple genes during the vascular procedure might constitute a promising approach to limit the response to vascular injury and improve patency rates.

INTRODUCTION

With over 200 million people affected worldwide, the global systemic disease burden of peripheral arterial disease remains substantial.[1] Simultaneously, an increasing number of revascularizations are performed in patients with significant occlusive disease.[2,3] Nevertheless, early and late failure due to recurrent narrowing (restenosis) after primarily successful procedures remains the major drawback of revascularization and limits the widespread utilization.

As surgical approaches yield comparable long-term results, understanding of genetic alterations that contribute to the pathophysiology of lumen loss and strategies aimed to modulate or suppress these genes is an appealing therapy.[4] Vascular remodeling, triggered by injury during balloon inflation or bypass graft implantation, results in differentiation and migration of smooth muscle cells as well as synthesis of extracellular matrix proteins that can lead to uncontrolled and excessive formation of intimal hyperplasia (IH).[5-7] During smooth muscle cell differentiation, (myo)fibroblasts undergo terminal modifications and express specific proteins to facilitate migration, reorganization of the cytoskeleton, and transmembrane signaling. These processes depend upon cell to cell as well as cell to extracellular matrix interactions, which are regulated by a network of cellular substrates.[8,9] Prior work has identified a complex signaling cascade with involvement of 100-1,000 genes differentially expressed during varying phases of vascular remodeling.[10] These data suggest the likely necessity to modulate, either by knockdown or overexpression, multiple genes instead of one to sufficiently hinder IH.

The current study corroborates previous findings, and sets out to simultaneously knockdown multiple genes that are involved in vascular remodeling.[11-14] Our goal is to target two genes that are not known to function in related pathways, and have been independently associated with IH. Thrombospondin-2 (TSP-2) is a matricellular protein implicated as a regulator of cell proliferation and angiogenesis.[15-17] Myristoylated alanine-rich C kinase substrate (MARCKS), a substrate of protein kinase C, is involved in the motility of various cell types, exerts pro-inflammatory actions, and increases proliferation.[18,19] With this study, we sought to elucidate the concept of dual gene knockdown of these two targets in an animal model and evaluate the response of vascular injury leading to intimal hyperplasia.

METHODS

Small interfering RNA and transfection agent

Rat-specific TSP-2 and MARCKS small interfering RNAs (siRNAs) were obtained from Ambion. Polyethylenimine (PEI, PolyPlus) was selected as an agent to mediate transfection. Apart from saline, all siRNA transfection solutions were combined with PEI. In previous published data, we demonstrated that the transfection agent alone or in combination with

non-coding RNA interference did not influence our genes of interest.[12-14] Hence, we did not include these treatment groups and selected saline as our control group.

Rat common carotid artery balloon injury model

The Institutional Animal Care and Use Committee at BIDMC approved this study and all animals were treated in compliance with the "Guide for the Care and use of Laboratory Animals" (8th edition, National Institutes of Health, 2010). A balloon injury of one common carotid artery was performed as previously described.[12] Briefly, male (400-450g) and female (275-300g) Wistar rats were anesthetized with isoflurane (2-3.5%). A 2F Fogarty catheter was introduced through an arteriotomy of the external carotid artery and advanced into the common carotid artery (CCA). The catheter was inflated and withdrawn with rotation to ensure complete circumferential denudation of the endothelium. This was repeated for a total of three passages. After removal of the catheter, the external carotid artery was canulated and the CCA was transfected with one of the following treatments for 15 minutes: 1. Saline, 2. siTSP-2, 3. siMARCKS, and 4. siTSP-2+siMARCKS.

Tissue harvesting and processing

Rats were euthanized at 24 hours and 21 days after surgery. Tissues were harvested using a protocol as previously described.[12] The denuded and transfected CCA was flushed with saline before harvest and stored in a fixative for 24 hours (formalin 10% at 4°C). The same procedure was performed for the contralateral non-denuded CCA, which served as a control and will be referred to as the non-denuded CCA. The tissue samples were embedded in paraffin. Subsequent analyses were performed in a double-blinded manner.

Reverse Transcriptase and Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted and purified from both the denuded and non-denuded CCA using a RNA purification kit (Qiagen). Quality of RNA was tested using a NanoDrop Spectrophotometer (Thermo Fisher Scientific). Subsequently, RNA was converted to cDNA using reverse transcription (Bio-Rad). Gene expression was quantified using Brilliant III SYBR® Green (Agilent Technologies) with Mx3005P QPCR system according to the manufacture's protocol. Custom oligos for TSP-2, MARCKS, and β_2-microglobulin were obtained from Integrated DNA Technologies and sequences are provided in Table S1. Each gene was normalized to β_2-microglobulin and carried out in duplicate. To determine the relative gene expression the $2^{-\Delta\Delta CT}$ method was used and all calculations are relative to their gene expression in the non-denuded CCA.

Histology

Transverse histological sections of 6μm were made from each segment and processed accordingly. Morphometry was performed using a video microscope. The ratio of the area of intima and media (I/M area ratio) was quantified after Verhoeff - van Gieson (American MasterTech Scientific) and six discontinuous sections were analyzed from each vessel.

Masson's Trichrome stain kit was used for the quantification of collagen deposition (American MasterTech Scientific), presented as percentage of the total area.

Immunohistochemistry

Heat mediated antigen retrieval was accomplished using proteinase K (Bio Rad). Information about specific antibodies and reagents used in this study are detailed in Table S2.
For protein expression standard immunofluorescence protocols were performed. Primary antibodies used for protein analysis were TSP-2 (Abcam), MARCKS (Santa Cruz), transforming growth factor-βeta (TGF-β) (Abcam), and matrix metalloproteinase-9 (MMP-9) (Abcam). Quantification of TSP-2, MARCKS, and TGF-β was based on an arbitrary scoring system and are presented as such. Staining density was scored accordingly: 1=minimal, 2=mild, 3=moderate, 4=strong, and 5=extensive. Dissemination was scored accordingly: 1=minimal staining distinctly localized to one layer, 2=limited staining blends into second arterial layer, 3=staining localized to two layers, 4=staining blends between two to three layers, 5=dissemination in all layers. Data for MMP-9 were presented as a percentage of the total intima area, due to the fact that staining was restricted to the intima only.
Proliferative cells were detected by staining with Ki-67 (Abcam). This was followed by exposure to 3,3'-diaminobenzidine (DAB) substrate buffer and counterstained with hematoxylin. Data were presented as positive Ki-67 cells per mm^2.
Total macrophage infiltration was assessed with CD68 pan-macrophage marker (AbD Serotec). Polarization was determined by CD68 co-staining with anti-iNOS (Abcam) or anti-CD206 (AbD Serotec) for M1 or M2 polarized macrophages, respectively. Standard immunohistochemical staining protocols were performed and macrophage data are presented as positive stained cells per mm^2. All analyses were performed by two blinded observers whom were given two-equally separated cross sections to quantify using NIH ImageJ v1.49 (National Institutes of Health).

Statistical Analysis

Samples within the same animal (denuded vs. non-denuded CCA) were analyzed with a paired sample t-tests. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test. Data were expressed as mean±standard deviation and statistical significance was considered when $P<.05$. All analyses were performed using IBM SPSS Statistics 23 (IBM Corp) and graphs were constructed using Prism6 (GraphPad).

RESULTS

mRNA levels of TSP-2 and MARCKS after arterial denudation

First, to validate our animal model, TSP-2 and MARCKS mRNA levels were compared between the denuded saline treated CCA and the non-denuded CCA at 24 hours and 21

days by qRT-PCR. All PCR data were normalized to the gene expression in the non-denuded CCA and expressed as a fold change. When the denuded saline treated CCA was compared to the non-denuded CCA at 24 hours, arterial denudation induced a significant 2.39-fold upregulation of TSP-2 mRNA (P=0.04) and a 1.93-fold upregulation of MARCKS mRNA (P=0.01) (Figure 1 A/C). After 21 days, TSP-2 mRNA levels were still significantly upregulated by 3.57-fold in the denuded saline treated CCA (P=0.02), whereas MARCKS mRNA levels were normalized to baseline (P=0.52) compared to the non-denuded CCA (Figure 1 B/D).

mRNA levels of TSP-2 and MARCKS after arterial denudation and siRNA transfection

Next, mRNA levels in all denuded CCAs were compared 24 hours after transfection. Compared to the saline treated CCA (2.40±1.04), TSP-2 mRNA levels were significantly reduced after siTSP-2 (0.77±0.23, P=0.01), siMARCKS (0.94±1.26, P=0.02), or siTSP-2+siMARCKS (0.25±0.13, P<0.001) treatment (Figure 1 A). Similarly, compared to the saline treated CCA (3.45±2.65), MARCKS mRNA levels were lower after siTSP-2 (1.14±0.53, P=0.048), siMARCKS (0.57±0.52, P=0.01), or siTSP-2+siMARCKS (0.63±0.34, P<0.01) treatment (Figure 1 C).

Twenty-one days after transfection, mRNA levels of our genes of interest were again evaluated. Compared to the saline treated CCA (3.73±2.51), TSP-2 mRNA levels remained significantly reduced after siMARCKS (1.10±0.99, P=0.03) or siTSP-2+siMARCKS (0.20±0.36, P<0.01) treatment, while there was a non-significant reduction after siTSP-2 (1.83±1.44, P=0.13) treatment (Figure 1 B). Compared to the saline treated CCA (1.27±1.04), MARCKS mRNA levels were not significantly different after siTSP-2 (0.97±0.58, P=0.96), siMARCKS (1.71±0.69, P=0.88), or siTSP-2+siMARCKS (2.03±1.84, P=0.58) treatment (Figure 1 D).

Protein expression of TSP-2 and MARCKS after arterial denudation and transfection

To determine the subsequent protein expression of TSP-2 and MARCKS, treatment groups were evaluated at 21 days by immunohistochemistry. Arbitrary scoring systems were used to score the density and dissemination of proteins in the CCAs. The validation of the animal models was again confirmed by comparing the denuded saline treated CCA to the non-denuded CCA. Protein expression of TSP-2 and MARCKS were significantly increased for both density and dissemination comparing the denuded to the non-denuded CCA and, therefore, validating the animal model (Figure 2).

Next, protein expression of all denuded CCAs was compared 21 days after transfections. Compared to saline treatment (3.47±1.28), TSP-2 protein density was significantly lower in siTSP-2 (1.20±0.21, P=0.02) treated CCA (Figure 2 A). Treatment with siTSP-2 also displayed lower TSP-2 protein density compared to siMARCKS (3.31±1.12, P=0.03) treatment. No other significant differences in TSP-2 protein density were found. When comparing denuded treatment groups for dissemination of TSP-2 protein, no significant differences were observed at 21 days (Figure 2 B).

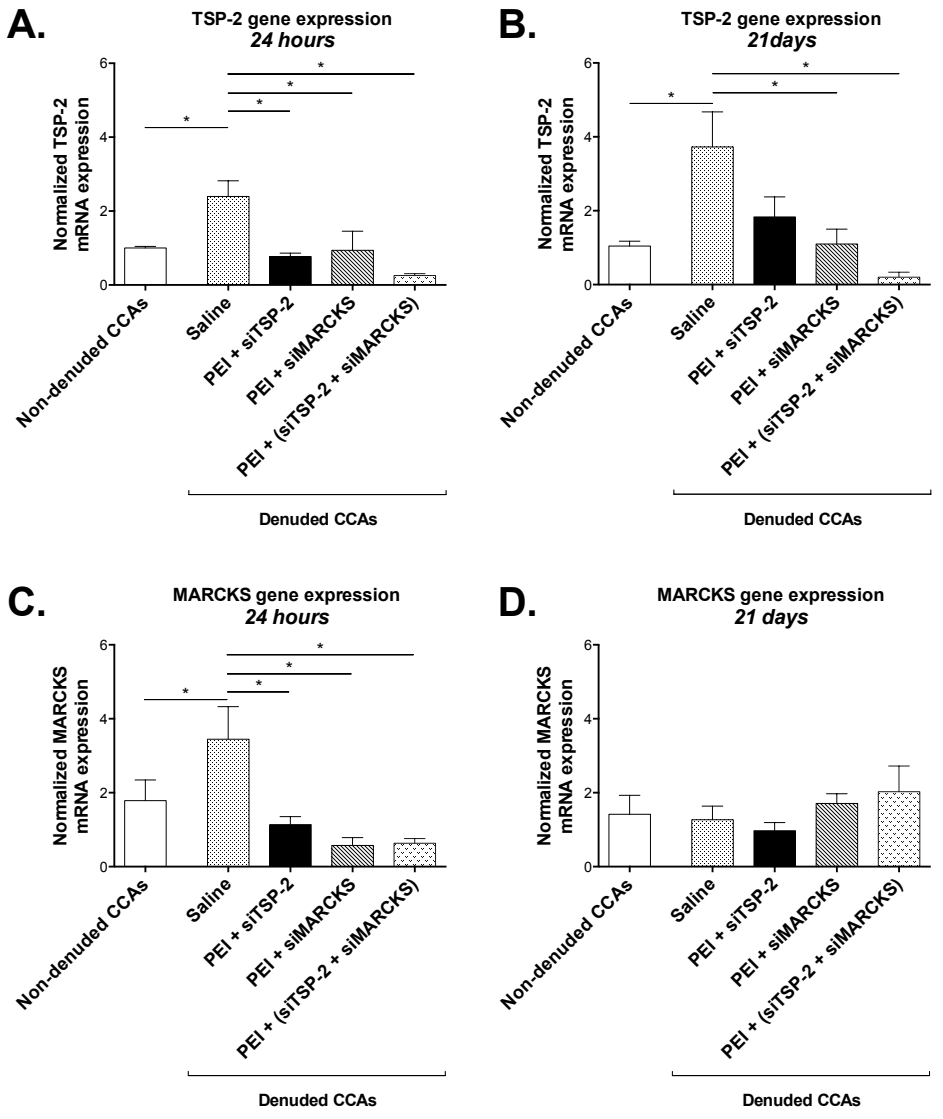

Figure 1. mRNA expression of TSP-2 at 24 hours (**A**) and 21 days (**B**). mRNA expression of MARCKS at 24 hours (**C**) and 21 days (**D**). Data are presented as mean ± S.E.M, * P<0.05.

Subsequently, MARCKS protein expression was compared between all denuded treatment groups 21 days after transfection. Compared to the saline treated CCA (3.08±0.58), density of MARCKS protein was significantly decreased after siTSP-2 (2.25±0.26, P=0.04), siMARCKS (1.73±0.24, P<0.01), or siTSP-2+siMARCKS (1.91±0.36,

P<0.01) treatment (Figure 2 C). Similarly, compared to the saline treated CCA (3.50±0.51), dissemination of MARCKS protein was significantly reduced with siTSP-2 (2.53±0.47, P=0.03), siMARCKS (1.75±0.40, P<0.001), or siTSP-2+siMARCKS (1.81±0.16, P<0.001) treatment (Figure 2 D).

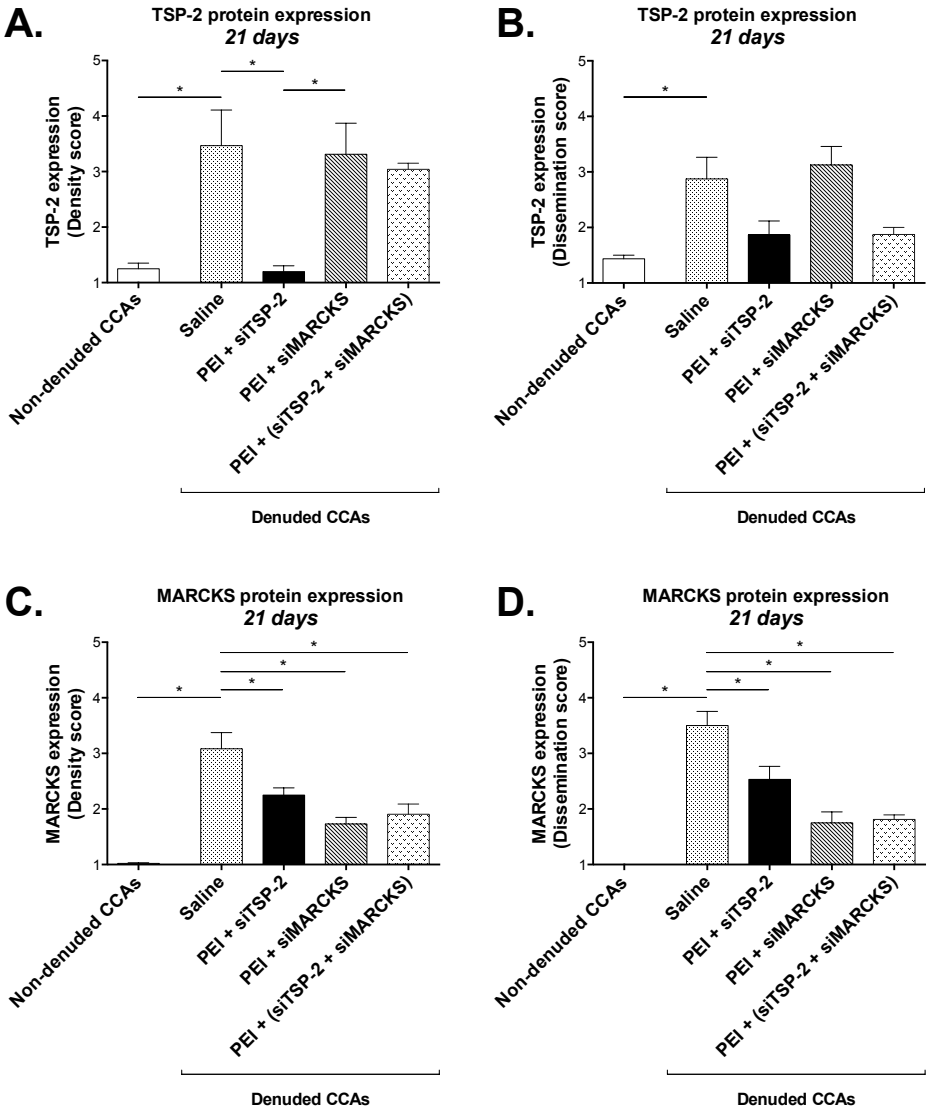

Figure 2. TSP-2 protein expression at 21 days: Density (**A**), Dissemination (**B**). MARCKS protein expression at 21 days: Density (**C**), Dissemination (**D**). Data are presented as mean ± S.E.M, * P<0.05.

Cell proliferation after arterial denudation and transfection

Cell proliferation is essential in vascular remodeling and intimal hyperplasia.[20] Therefore, representative cross-sections were stained with anti-Ki67 to examine cell proliferation after 21 days and data are expressed as proliferating cells/per mm^2. Compared to the non-denuded CCA (13.4±2.7), cell proliferation was significantly increased in the denuded saline treated CCA (33.8±10.0, P=0.02). These results served as a validation for our animal model (Figure 3 A).

Cell proliferation was subsequently compared between all denuded treatment groups at 21 days. Compared to the saline treated CCA (33.8±10.0), cell proliferation was significantly less with siTSP-2 (14.4±5.1, P<0.01), siMARCKS (6.7±6.3, P<0.001), or siTSP-2+siMARCKS (4.0±2.4, P<0.001) treatment (Figure 3 A).

Collagen deposition after arterial denudation and transfection

Deposition of collagen in the arterial wall was determined after 21 days and data were expressed as percentage stained/per mm^2. Compared to the non-denuded CCA (10.2±2.5), collagen deposition was significantly increased in the denuded saline treated CCA (47.0±4.0, P<0.001) (Figure 3 B).

Collagen deposition was also compared between all denuded treatment groups at 21 days. Compared to the saline treated CCA (47.0±4.0), significantly less collagen was deposited after treatment with siTSP-2 (32.2±1.0, P<0.001), siMARCKS (31.4±3.6, P<0.001), or siTSP-2+siMARCKS (30.8±4.5, P<0.001) (Figure 3 B).

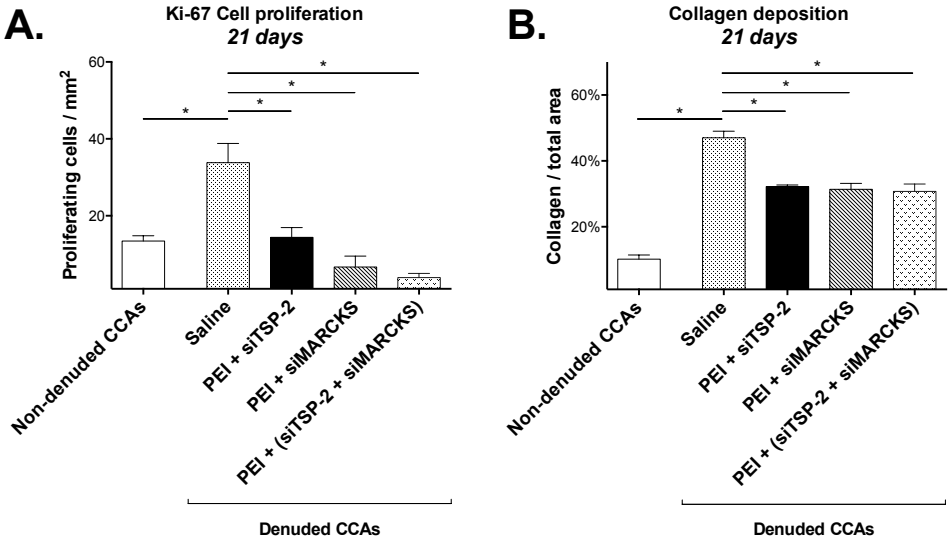

Figure 3. Ki67 cell proliferation (**A**), and collagen deposition (**B**) at 21 days. Data are presented as mean ± S.E.M, * P<0.05.

Macrophage infiltration after arterial denudation and transfection

Macrophage infiltration contributes to intimal hyperplasia and vascular inflammation.[21] Hence, CCAs were examined for macrophage infiltration at 21 days by anti-CD68 staining. Data are presented as positive CD68 cells/per mm^2. As detailed in Figure 4 A, macrophages were significantly higher in the denuded saline treated CCA (223.4±146.3) compared to the non-denuded CCA (12.5±7.6, P=0.03).

Twenty-one days after transfection all denuded treatment groups were analyzed and compared to each other. Compared to the saline treated CCA (223.4±146.3), the number of macrophages was not significantly altered after treatment with si-TSP-2 (232.0±84.5, P>0.99). Although macrophage infiltration was lower after siMARCKS (111.6±27.0, P=0.09) or siTSP-2+siMARCKS (139.9±70.1, P=0.29) treatment, these differences were not statistically different compared to treatment with saline (Figure 4 A).

M1 and M2 positive macrophages after arterial denudation and transfection

While no significant differences in total macrophage infiltration were found within the denuded CCA treatment groups, we were interested in the specific polarization of these activated macrophages. Hence, we calculated the ratio of M1 and M2 activated macrophages at 21 days after transfection in all denuded treatment groups. Data are expressed as a ratio.

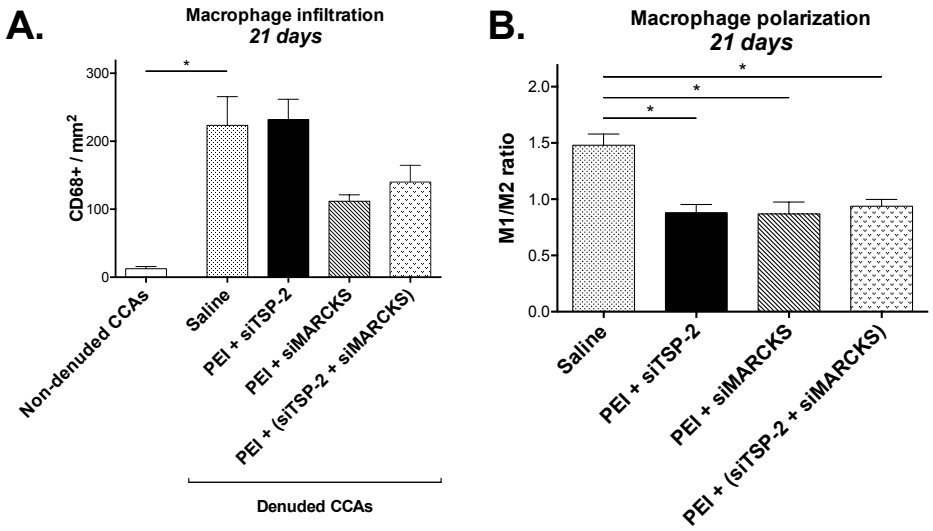

Figure 4. Macrophage infiltration (**A**), and macrophage polarization (**B**) at 21 days. Data are presented as mean ± S.E.M, * P<0.05.

Twenty-one days after transfection the M1/M2 ratio after saline treatment was above one (1.48±0.28), which reflects more pro-inflammatory M1 activated macrophages in the arterial wall. Compared to the saline treated CCA, all siRNA treated groups displayed a ratio less than one and thus significantly lower, including siTSP-2 (0.88±0.20, P<0.001), siMARCKS (0.87±0.30, P<0.001), or siTSP-2+siMARCKS (0.94±0.17, P<0.001) treatment (Figure 4 B). This indicates more anti-inflammatory M2 activated macrophages in all siRNA treated groups.

MMP-9 and TGF-β expression after arterial denudation and transfection

As MMP-9 and TGF-β are considered to be major downstream signaling proteins in intimal hyperplasia, immunofluorescence staining was performed at 21 days to examine the protein expression of MMP-9 and TGF-β. The percentage positive stain/per mm^2 of the intima was used to measure MMP-9, whereas arbitrary scores were utilized to quantify the density of TGF-β protein. In response to arterial denudation, both MMP-9 and TGF-β expression were increased at 21 days when comparing the denuded saline treated CCA to the non-denuded CCA (Figure 5).

Twenty-one days after transfection MMP-9 expression was determined in all denuded treatment groups. Compared to the saline treated CCA (64.9±5.9), MMP-9 expression was significantly reduced after treatment with siTSP-2 (37.7±5.3, P<0.001), siMARCKS (44.6±8.1, P<0.01), or siTSP-2+siMARCKS (43.6±2.2, P=0.001) (Figure 5 A).

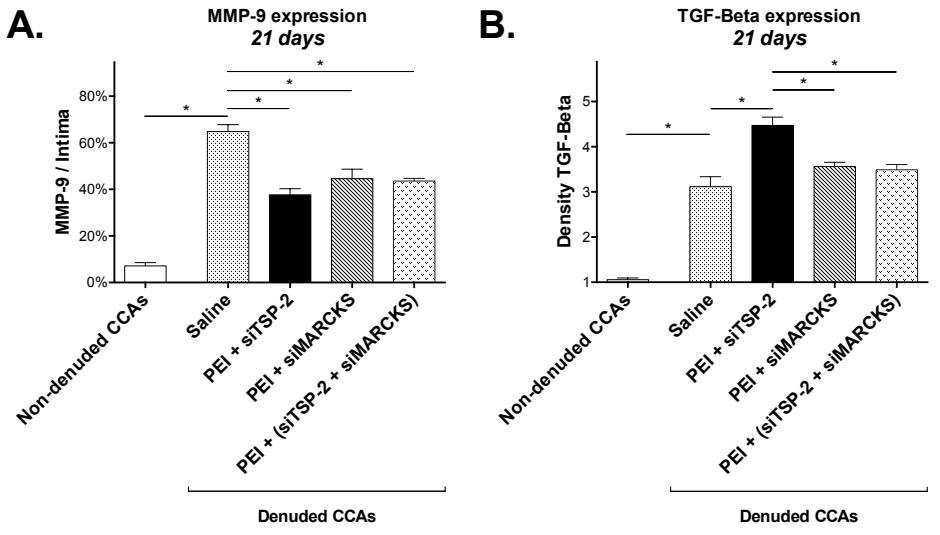

Figure 5. Protein expression of matrix metalloproteinase-9 (MMP-9) (**A**) and transforming growth factor-βeta (TGF-β) (**B**) at 21 days. Data are presented as mean ± S.E.M, * P<0.05.

In addition, TGF-β expression was compared between all denuded treatment groups 21 days after transfection. Compared to the saline treated CCA (3.11±0.45), the expression of TGF-β was increased after siTSP-2 (4.47±0.32, P<0.001), while no significant differences were found compared to siMARCKS (3.56±0.18, P=0.24) or siTSP-2+siMARCKS (3.49±0.24, P=0.37) treatment. Of note, TGF-β expression was significantly higher after siTSP-2 compared to siMARCKS (P=0.01) or siTSP-2+siMARCKS (P<0.01) treatment (Figure 5 B).

Intima/media area ratio after arterial denudation and transfection

Twenty-one days after arterial denudation, the degree of intimal hyperplasia was evaluated morphologically and quantitatively. Data are expressed as I/M area ratios. As presented in Figure 6, the animal model was again validated by our data indicating a significant increase in I/M area ratio in the denuded saline treated CCA (1.29±0.41) compared to the non-denuded CCA (0.05±0.003, P<0.001).

Finally, the I/M area ratio was compared between all denuded treatment groups at 21 days. Compared to the saline treated CCA (1.29±0.41), the I/M area ratio was lower after treatment with siTSP-2 (1.22±0.62, P=0.99), siMARCKS (0.97±0.49, P0=0.61), or siTSP-2+siMARCKS (1.00±0.44, P=0.70), however, these differences were not statistically significant (Figure 6).

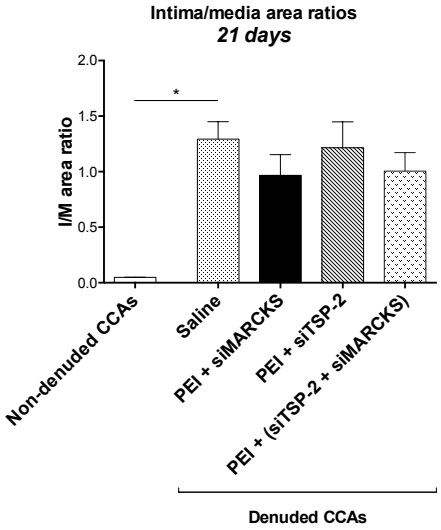

Figure 6. Intima/media area ratio at 21 days. Data are presented as mean ± S.E.M, * P<0.05.

DISCUSSION

Remodeling after vascular interventions is modulated by numerous of signaling pathways and any deregulation may promote intimal hyperplasia and restenosis. While large amounts of data from *in vitro* studies have been published on this topic, we lack complete understanding of the underlying cellular and molecular mechanisms *in vivo*. In this study, we investigated the role of dual knockdown of TSP-2 and MARCKS on the regulation of vascular remodeling after arterial injury by means of a rat model.

The feasibility of multiple gene silencing has been demonstrated before in a cell culture of human vascular smooth muscle cells and endothelial cells.[11] Multiple gene knockdown *in vitro* resulted in a modest decrease of silencing efficacy compared to the RNA levels after single siRNA treatment. Contrary to the above-mentioned study, our data indicated that, after dual knockdown, TSP-2 and MARCKS mRNA levels were lower than mRNA levels after single gene knockdown, suggesting that these seemingly unrelated pathways interconnect. These findings support the notion of silencing multiple genes *in vivo* without losing efficacy when utilizing siRNAs simultaneously.

Thrombospondin-2 modulates the cell to extracellular matrix interactions and has been associated with a variety of biologic functions, including regulation of migration, adsorption, differentiation, and apoptosis.[16,22,23] It has been demonstrated that TSP-2 contains several promotor regions for MMPs and transcription factors, including nuclear factor kappalight-chain-enhancer of activated B cells (NF-kB).[24] Modulation of these transcription factors, along with alterations of TGF-β by TSP-2, may induce M2 macrophage polarization, indicative of a state of anti-inflammatory repair and remodeling.[25,26] Moreover, inhibition of TSP-2 resulted in reduced cell proliferation that may be suggestive of a better organized extracellular matrix. This is in line with previous studies demonstrating that TSP-2 promotes proliferation of human aortic smooth muscle cells.[27,28] Others have found that TSP-2 antagonizes endothelial cell migration and proliferation, which prevents re-endothelialization after vascular injury that subsequently may trigger chronic thrombogenic events.[17] Therefore, lower TSP-2 levels, as achieved by siRNA, exert both beneficial features on smooth muscle as well as endothelial cells to efficiently target restenosis.

MARCKS mediates protein kinase C signaling through phosphorylation and subsequent modification, and may be involved in cell growth via filamentous actin and calmodulin dependent cell functions.[18,19] In prior studies, we showed that MARCKS silencing arrested smooth muscle cell differentiation and inhibited migration through upregulation of cyclin dependent kinase inhibitor $p27^{kip1}$ *ex vivo*, without affecting endothelial cells.[14] This was confirmed by Yu et al., who demonstrated that the inhibition of proliferation likely acts through $p27^{kip1}$ and KIS (kinase interacting with stathmin) dependent pathways.[29] Importantly, MARCKS knockdown has been shown to inhibit smooth muscle cell but induce endothelial cell proliferation and re-endothelialization. This functional selectivity makes MARCKS an appealing target to attenuate restenosis. Interestingly, a prospective cohort study of 204 patients undergoing bypass demonstrated that the $p27^{kip1}$ gene (homozygous

for the minor variant A allele) was associated with a 2.5-fold reduction of treatment failure and restenosis.[30] Therefore, MARCKS-mediated modulation of these cell cycle regulators may have a potential role as a determinant in better clinical outcome.

The current study extends previous findings and suggests variability of gene silencing. Our data revealed that MARCKS knockdown specifically decreases TSP-2 mRNA levels, which suggests a common alternative signaling pathway. The common denominator may be Rac1, a Rho family GTPase that controls cytoskeletal actin reorganization. MARCKS knockdown appears to prevent activation of Rac1, which is an essential component for actin polymerization and smooth muscle cell migration.[31] Prior studies in human endothelial cells demonstrated that Rac1 mediates activation of TSP-2.[32] This suggests that Rac1 is a signaling pathway connecting the functional capacities of TSP-2 and MARCKS, although more research is needed to delineate these mechanisms of action.

Despite the various modulating effects after dual gene knockdown of TSP-2 and MARCKS, we could not demonstrate a significant reduction in I/M area ratios. This highlights the complexity of the vascular microenvironment and implicates numerous of signaling pathways participating in the pathogenesis of intimal hyperplasia. However, our data support the idea of multiple gene silencing to effectively reduce neointima thickening and stresses the need to identify other high-profile genes and target alternative pathways simultaneously.

CONCLUSIONS

This study demonstrates efficient dual gene silencing of TSP-2 and MARCKS through a constrained 15-minute transfection of siRNA in an animal model. These results support favorable effects on vascular remodeling by interfering with alternative and complementary pathways simultaneously. Multiple gene silencing appears ideally suited for vascular interventions with direct local delivery of a mixture of siRNAs to the tissues and cells of interest.

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SUPPLEMENTARY

Table S1. Primers (Integrated DNA Technologies, Coralville, IA, USA)

Target	Primer sequence
TSP-2	Forward: 5′- GGACAACTGCAGGCTTGTGTTCAA – 3′
	Reverse: 5′- AGTCTGTCTCGGTGATGGCATTGT – 3′
MARCKS	Forward: 5′- TTGTTGAAGAAGCCAGCATGGGTG – 3′
	Reverse: 5′- TCTCCTGCCCATTTGCTTTGGAAG – 3′
β_2-microglobulin	Forward: 5′ - ACACTGAATTCACACCCACCGAGA – 3′
	Reverse: 5′ - TGATTACATGTCTCGGTCCCAGGT - 3′

Table S2. List of Reagents

siRNA		
Reagent	**Catalog Number**	**Company**
Silencer® Select Thbs-2 siRNA	s146414	Thermo Scientific Inc, Waltham, MA, USA
Costum Ambion® MARCKS siRNA	4457297	Thermo Scientific Inc, Waltham, MA, USA
In vivo jetPEI® delivery reagent	201-10G	Polyplus, Illkirch, France
qRT-PCR		
Reagent	**Catalog Number**	**Company**
RNeasy FFPE Kit	73504	Qiagen, Hilden, Germany
RNase-Free DNase Set	79254	Qiagen, Hilden, Germany
IScript cDNA Synthesis Kit	1708891	Bio-Rad, Hercules, CA, USA
Brilliant III SYBR® Green master mix	600882	Agilent Technologies, Santa Clara, CA, USA
Histology		
Reagent	**Catalog Number**	**Company**
Verhoef-van Giesson stain	KTVEL	American MasterTech Scientific, Lodi, CA, USA
Masson's Trichrome stain	KTMRT	American MasterTech Scientific, Lodi, CA, USA
Protein Expression		
Protein	**Primary Antibody Catalog Number, Company**	**Secondary Antibody, Catalog Number, Company**
TSP-2	ab84469, Abcam, Cambridge, MA, USA	Cy5 ab6719, Abcam, Cambridge, MA, USA
MARCKS	SC6454, SantaCruz, Dallas, TX, USA	Alexa Fluor 488 705-545-147, Jackson ImmunoResearch, West Grove, PA, USA
TGF-β	ab66043, Abcam, Cambridge, MA, USA	Cy5 ab6719, Abcam, Cambridge, MA, USA
MMP-9	ab38988, Abcam, Cambridge, MA, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA
Cell Proliferation		
Reagent	**Catalog Number**	**Company**
Ki67	ab1667	Abcam, Cambridge, MA, USA
Macrophage Infiltration		
Antibody	**Primary Antibody Catalog Number, Company**	**Secondary Antibody, Catalog Number, Company**
CD-68 (pan macrophage marker)	MCA341GA, AbD Serotec, Raleigh, NC, USA	Alexa Fluor 488 715-545-150, Jackson ImmunoResearch, West Grove, PA, USA
iNOS (M1 marker)	ab136918, Abcam, Cambridge, MA, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA
CD206 (M2 marker)	MCA2155T, AbD Serotec, Raleigh, NC, USA	Alexa Fluor 594 ab150084, Abcam, Cambridge, MA, USA

CHAPTER 11

SUMMARY, GENERAL DISCUSSION, AND FUTURE PERSPECTIVES

SUMMARY

In *Part I*, the current performance of lower extremity revascularization as well as risk factors for treatment failure were assessed. In **Chapter 2**, we evaluated the comparative effectiveness of the initial revascularization strategy in patients without prior ipsilateral vascular interventions. Overall, early morbidity, but not mortality, was substantially lower after an endovascular-first compared to a bypass-first procedure, at the cost of higher rates of postoperative secondary revascularizations. We found that older patients and those with more severe comorbidities and infrapopliteal disease were more likely candidates to undergo an endovascular-first approach. **Chapter 3** provides insight into the profound effect of diabetes on long-term outcomes after revascularization. Insulin-dependent diabetes was shown to heighten the risk of incomplete wound healing, restenosis, and major amputation as compared to no diabetes, while noninsulin-dependent diabetes was not associated with these long-term adverse events. These data further suggest that the major adverse events associated with insulin-dependent diabetes may be most mitigated in patients who are appropriately selected and anatomically suitable for a bypass. **Chapter 4** evaluated long-term outcomes of renin-angiotensin system inhibitor use in patients with CLTI undergoing revascularization. Discharge on renin-angiotensin system inhibitors was associated with lower periprocedural cardiovascular adverse events, as well as a 30% improved survival up to five years after revascularization. The benefit of renin-angiotensin system inhibitors was restricted only to patients on a high-dose therapy. In **Chapters 5 and 6**, we introduce two underexposed risk factors in patients with CLTI undergoing bypass, namely preoperative anemia and blood transfusions. **Chapter 5** demonstrates that early mortality, major amputation, and cardiovascular adverse events are inversely associated with preoperative hematocrit levels, with the highest event rates in the most severely anemic patients. Blood transfusions, or the perceived need for transfusion due to bleeding, did not reduce – but rather increased – postoperative risks in the less severely anemic patients. In line with these findings, **Chapter 6** was dedicated to assess long-term outcomes of preoperative anemia. More than half of patients with CLTI present with anemia and both late mortality and major amputation were increased by two-fold among patients with preoperative anemia. Blood transfusions were again strongly correlated with anemia and associated with higher in-hospital and late postoperative adverse events in selected patients with CLTI. **Chapter 7** revealed that the incidence of unplanned readmissions within 30 days after endovascular intervention was relatively common for CLTI (1 in 6 patients) and claudication (1 in 15 patients). Mortality and morbidity after index admission were significantly higher for those readmitted. In both patients with CLTI and claudication, risk factors for unplanned readmission included patient characteristics and procedure-related factors, as well as occurrence of in-hospital complications such as bleeding and unplanned return to the operating room. The success of revascularization can be threatened by the occurrence of restenosis or occlusion. **Chapter 8** revealed that prior ipsilateral revascularizations are not risk free and impact the subsequent bypass procedure. Prior

revascularization proved to be an important risk factor for early major adverse limb events, reinterventions, and unplanned reoperations. Additionally, bypass after a prior endovascular intervention was associated with wound infections, whereas early reinterventions were more commonly performed following bypass after a prior bypass procedure.

Restenosis after revascularization remains a challenging clinical complication. In *Part II*, we aimed to further corroborate previous findings and develop novel gene therapeutic strategies in preclinical studies to ameliorate factors that contribute to early and late lumen loss, wich may serve as a foundation to effectively improve patency rates after revascularization. In **Chapter 9,** we introduced a novel delivery and transfection method of a gene-based therapy in a rat model. In a validated balloon angioplasty injury model, effective transfection of cells in the injured wall was demonstrated only after a 15-minute time frame of local intraluminal transfusion. The therapeutic strategy involved non-viral delivery of RNA interference targeted against thrombospondin-2, a matricellular protein, which ameliorated vascular remodeling by inhibition of major contributing proteins, as well as cell proliferation, collagen deposition, and the inflammatory response. Encouraged by these results, we set out to selectively target multiple genes involved in intimal hyperplasia in **Chapter 10**. Similarly, we validated our transfection method by selective knockdown of our genes of interest (thrombospondin-2 and myristolated alanine-rich C kinase substrate [MARCKS]) through a short transfection period of 15 minutes. As a result, effective gene silencing was still measured after 21 days. Simultaneous knockdown of two different pathways involved in intimal hyperplasia decreased cellular proliferation even further than selective knockdown of these genes separately. Furthermore, long-term gene silencing induced an anti-inflammatory M2-type response supported by alterations of transforming growth factor-βeta. These data show that the application of RNA interference is not only a promising technique to identify gene function *in vivo*, but also may be an effective therapeutic tool to target intimal hyperplasia and restenosis.

DISCUSSION AND FUTURE PERSPECTIVES

Revascularization strategies for PAD have significantly evolved over the past decades. Until recently, treatment of PAD primarily consisted of peripheral arterial bypass surgery with a vein or prosthetic conduit. As an alternative to arterial bypass, catheter-based therapies or endovascular interventions, such as balloon angioplasty with or without stent, are now more commonly utilized. Currently, more than three endovascular interventions are performed for every one bypass, and the total number of lower extremity revascularizations almost doubled in the last decade.[1-3] Parallel to this paradigm shift, a trend toward lower amputation rates is observed, although there is a higher need of reinterventions after endovascular procedures. Together with multiple others[4-6], our retrospective cohort study demonstrated perioperative benefits of an endovascular-first over a bypass-first strategy

in terms of adverse cardiovascular events and unplanned readmissions, at the cost of higher postoperative secondary revascularizations (Chapter 2). It is unlikely that the increase of endovascular interventions is merely the result of more reinterventions after a primary endovascular approach, and additionally a causal relationship related to the change in amputation rates cannot be established. Due to the less invasive nature of these techniques, which simultaneously lowers the threshold for intervention, patients that were previously deemed unfit for surgical bypass may now be receiving endovascular revascularization. Similarly, patients with claudication whom previously deferred surgery because of the heightened morbidity and mortality risk of the procedure itself may now be eligible for endovascular interventions. Overall, the use of endovascular intervention has led to an increase in the proportion of PAD patients now treated with a revascularization procedure.

Based on the available literature it is not easy to defend either an endovascular- or bypass-first strategy. The initial choice of treatment largely depends on patient- and procedure-specific factors, the severity and extent of arterial disease, the availability of a suitable vein, and vascular anatomy.[7] Patient-centered care is essential since all these characteristics may vary per patient, which subsequently influences the potential success of endovascular or surgical procedures. However, current evidence to guide decision-making is scarce and limited to few high-quality randomized, controlled trials.[4,8] Attempts to compare outcomes between revascularization strategies have been deterred by inconsistencies of definitions, considerable heterogeneity of clinical presentation, and endpoints that were clinically less relevant.[9-11] This is reflected by the lack of recommendations by vascular society practice guidelines.[7,12-14] The most prominent trial, the BASIL trial, prospectively compared the effectiveness of angioplasty to open bypass in patients with severe ischemia and reported lower early morbidity after angioplasty but no differences between treatment groups with respect to amputation-free survival at one- and three-years.[4] Nevertheless, patients allocated to the bypass group, surviving more than 2 years, had lower overall mortality (hazard ratio [HR] 0.61, 95% CI [0.50 – 0.75]).[15] Moreover, patients with a prosthetic bypass fared worse compared to those undergoing a bypass with vein conduit and the reintervention rate after angioplasty was 11% higher than after bypass. Due to several issues in study design, the real-world generalizability of this trial was limited and the remaining evidence is fueled by population-based observational studies. These studies support the idea of favorable perioperative outcomes after endovascular intervention but a higher rate of restenosis compared to bypass, even though some showed conflicting results.[5,16-25] As with coronary interventions, this may be perceived as an acceptable tradeoff in those with multiple life-limiting comorbidities and a relatively short life expectancy.

It is important to realize that a failed prior ipsilateral procedure affects future interventions and to recognize inferior outcomes with repeated procedures. Some adopted an endovascular-first strategy, however, this should not be considered a free pass.[26,27] Increasing evidence suggests that subsequent revascularization procedures have a worse

prognosis than the initial intervention.[28-34] Not only did we demonstrate a higher risk of early major adverse limb events and reinterventions, we showed that these adverse events were consistently associated with both prior ipsilateral endovascular intervention as well as bypass (Chapter 8). The increased risks of adverse events following secondary revascularization may be explained by a more aggressive disease process. Patients who have already suffered failure of a primary procedure are likely to represent a selected group that is at greater general risk for treatment failure and other adverse outcomes. However, as restenosis is a major clinical concern after revascularization, critical evaluation of interventions and their outcomes is essential. Upcoming randomized, controlled trials investigating novel devices and factors influencing treatment success are a much-needed addition to the current literature.[35,36] Meanwhile, opportunities may lie in preoperative risk scores, perhaps multifactorial decision models, and stratifying patients based on systemic risk factors and severity of disease.

Major impediments to improving the care of patients with PAD are related to the lack of disease recognition, poor understanding of factors that influence patient outcomes, and the gross underuse of safe and effective secondary preventative measures. The PARTNERS (PAD Awareness, Risk and Treatment: New Resources for Survival) study screened just under 7,000 people on PAD in a primary care setting.[37] Two of their extrapolated conclusions were that, first, PAD (defined as ankle-brachial index of less than 0.9) was poorly recognized – 44% of cases were diagnosed after enrollment to the study – and, second, risk factors in patients with PAD were insufficiently managed, including hyperlipidemia, hypertension, and antiplatelet therapy.

Risk stratification may be a useful tool in identifying those at higher risk that may benefit from adequate allocation of necessary care and stricter follow-up after revascularization.[11,38,39] Along those same lines, Chapter 3 to 7 shed more light on conventional and more underexposed risk factors. By distinguishing diabetes type, we found that insulin-dependence is associated with the greatest risk of major adverse outcomes and restenosis after revascularization; however, these adversities were most mitigated by bypass as opposed to endovascular intervention (Chapter 3). The findings of the present thesis indicate variability exists in the role of preoperative risk factors for each of the treatment strategies, which suggests that different patient factors affect outcomes depending on the type of revascularization. Precise risk stratification has become important in order to improve clinical decision-making and to determine the most appropriate path of care (Chapter 7). To address this need, the Society of Vascular Surgery developed a new classification system for CLTI to stratify risk based on three major categories: wound, ischemia, and foot infection (WIfI).[11] This novel scoring system appears to provide a more comprehensive clinical staging stratification, which allows for more meaningful comparisons between revascularization strategies and better predictions of wound healing and amputation risk.[40-42]

Within two large vascular registries, we showed the marked impact of preoperative anemia in patients with the most advanced stages of PAD. As anemia is often perceived as a mere consequence of aging and a marker of disease, it is rarely treated preoperatively.[43,44] This is reflected by the excessive high proportion that presents with anemia prior to surgery (57-70%). As in other surgical specialties[45], patients with preoperative anemia undergoing revascularization have a higher risk of early and late adverse outcomes (Chapter 5 and 6). Since postoperative correction of anemia with blood transfusions was not free of risk, management prior to the surgical incision may be appropriate whenever possible. This apparent contraindication stresses the need for future studies investigating an optimal threshold for blood product usage in patient with PAD. Ultimately, hemoglobin or hematocrit levels are not simply laboratory values but rather modifiable risk factors, and interpretation as well as correction before surgery may be the key to successful management. Measures and efforts to devise best practices have been bundled in a concept, known as "patient blood management," which aims to change care paradigms from the perspective of transfusion as a therapy to addressing the underlying clinical condition, even for anemia of chronic disease.[46-48]

The relatively high contribution of risk factors, such as cardiovascular disease and diabetes, toward mortality in PAD patients suggests that emphasis on aggressive risk factor modification is justified. Since a wide spectrum of clinicians from primary care providers, cardiologists, interventionalists, to vascular surgeons serve the PAD population, increased attention to medication compliance as well as implementation of intensified secondary prevention is an appealing target for the future. Consistent with this rationale, physician adherence to guideline-recommended medical management for patients with PAD is low, with underuse of cardioprotective medications.[49-54] Although adherence to secondary prevention guidelines seems to improve over time, the implications on overall cardiovascular risks are likely underappreciated. Change in current prescribing patterns would likely benefit a large number of patients with PAD, as was demonstrated in Chapter 4 where an appropriate dose of renin-angiotensin system inhibitor lowered long-term mortality by 30%. A recent study modeled the potential effectiveness of optimizing three medical therapies – antiplatelet agents, statins, and angiotensin-converting enzyme inhibitors with benchmark adherence targets of 85% for each therapy – and concluded that guideline-recommended medical management in the PAD population would prevent 212,166 cardiovascular adverse events in North America and Europe alone.[55] While this particularly vulnerable population appears to benefit from optimization of cardiovascular health, optimal medical management has also been associated with better outcomes of lower extremity vasculature and revascularization, which provides further opportunities to improve overall patient care.[51,56-58]

The prospect that inhibition of complex cellular and molecular processes involved in the pathophysiology of restenosis may significantly influence limb-related outcomes after

revascularization has sparked excitement. The preclinical studies described in this thesis aim to provide convincing evidence of the broad potential of gene therapeutic approaches feasible under clinically applicable conditions within the constraint of the operating room, setting the stage for future studies. Revascularization lends itself particularly well for localized application of gene therapy because it can be delivered *ex vivo* to conduits or can be specifically isolated to the post-angioplasty lesions with drug-eluting stents, notably without unintended systemic side effects or transfection of off-target cells. Intraluminal delivery used in this thesis appears to be an effective delivery approach, transfecting the cells closest to the injury site (Chapter 10 and 11). Nevertheless, a strategy of intraluminal combined with perivascular transfection, for instance with a biodegradable cryogel polymer containing RNA interference, resulting in gene transfer to the adventitia as well as to cells comprising the inner lining of the lumen, may hold even greater promise.

It is essential to realize that restenosis is a complex mechanism of a multitude of genes and pathways that may vary over time according to the different stages of vascular remodeling. To this end, knockdown or overexpression of a single gene is likely insufficient to stall intimal hyperplasia. Previous work showed the comprehensive genomic response to vascular remodeling with 100-1,000 genes differentially expressed at various time points in endothelial and smooth muscle cells.[59] Therefore, efficiently interfering in different pathways simultaneously has great potential to effectively inhibit restenosis, and ongoing work will build on the foundations established in Chapter 10.

Nevertheless, significant advances are required before these described approaches can be implemented and realized in humans. The first major challenge is the delivery technique of gene-based therapies. Delivery materials are required to transport RNA interference to the cells of target tissue and subsequently function as a transfection agent to mediate efficient intracellular transportation. Unmodified RNA interference is unstable, non-specific, and incapable of crossing cell membranes. Justified concerns have been raised regarding the safety of viral delivery vectors that may elicit an unwanted immune response.[60,61] Even though studies in the current thesis applied non-viral delivery, off-target effects and cell toxicity of the transfection agent utilized in our models have been described.[62] To surmount this challenge, a large diversity of vehicles and chemical modifications are under exploration to improve uptake and efficacy.[61,63-66] Nanomedicine and nanoparticles may be promising avenues, as they specifically bind to ligands of target cells in a highly complex and heterogeneous microenvironment, while minimizing similar interactions to non-target cells.[67,68] Continued research into the numerous delivery platforms will help elucidate these processes. Second, to properly assess the promise of preclinical studies, endpoints in animal experiments must correlate with those used in clinical studies. Meaningful patient-centered endpoints, such as critical restenosis and reduced flow, need for revascularization, or systemic inflammation, are more pragmatic outcomes than only evaluating vessel wall thickness. In addition, widespread translation to clinical application may be hampered by

the extent to which animal models accurately represent the molecular mechanisms in humans. As a result, more work to define the exact pathogenesis of intimal hyperplasia must be carried out in the near future.

In conclusion, through a critical review of perioperative and long-term prognosis after lower extremity revascularization, this thesis has contributed to a better understanding of risk factors and patient performance following open and endovascular surgery. Herein, we have alluded to the potential of gene therapy, not only as a method of localized and selective knockdown of genes, but also as a platform for disease-specific treatment of restenosis. As these therapies become more commonplace, it is likely that they will have a significant impact on future revascularizations.

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APPENDICES

SUMMARY IN DUTCH - NEDERLANDSE SAMENVATTING
REVIEW COMMITTEE
ACKNOWLEDGEMENTS
CURRICULUM VITAE

SUMMARY IN DUTCH - NEDERLANDSE SAMENVATTING

Perifeer arterieel vaatlijden is een manifestatie van gegeneraliseerde atherosclerose in de arteriën van de onderste extremiteiten. Door deze vernauwing of zelfs afsluiting kan minder of geen bloed doorstromen naar de benen waardoor zuurstofgebrek ontstaat. Naast acuut obstructief vaatlijden worden de meeste symptomen veroorzaakt door een chronische en progressieve vorm. De symptomen zijn afhankelijk van de ernst van de vernauwing(en) en ischemie (zuurstoftekort) waarin onderscheid wordt gemaakt tussen claudicatio intermittens en kritische ischemie. Bij claudicatio intermittens (ook wel etalagebenen genoemd) ontstaat een krampachtige pijn in de beenspieren tijdens het lopen, die na rust volledig verdwijnt en na inspanning opnieuw ontstaat. Dit kan tot een sterke beperking van de dagelijkse activiteiten leiden. Bij een verder gevorderd stadium, kritische ischemie, is er sprake van pijnklachten in rust en/of weefselverlies waarbij wonden ontstaan aan de voeten en benen. Naast een hoger risico op amputaties van de onderbenen, hebben deze patiënten vaker meer risicofactoren, zoals diabetes mellitus, nierinsufficiëntie en andere hart- en vaatziekten, waardoor de kans op overlijden veel hoger ligt. Indien progressie van de klachten of bij patiënten met kritische ischemie is een chirurgische ingreep noodzakelijk om de bloedvoorziening te verbeteren, ook wel revascularisatie genoemd. Behandelingsmogelijkheden omvatten een endovasculaire interventie, oftewel een dotterbehandeling met of zonder stent waarbij een ballonkatheter wordt ingebracht via de liesslagader om de vernauwing op te rekken, of bypasschirurgie waarbij het vernauwde traject wordt overbrugd met een eigen ader of kunststofprothese. Voor dit proefschrift hebben wij de resultaten van revascularisatie voor perifeer arterieel vaatlijden geëvalueerd, maar ook nieuwe strategieën ontwikkeld om de prestatie van deze chirurgische behandelingen te verbeteren.

In *Deel I* wordt de huidige prestatie van revascularisatie procedures voor perifeer arterieel vaatlijden evenals de risicofactoren die bijdragen aan het falen van deze behandeling bestudeerd. In **hoofdstuk 2** worden twee primaire revascularisatie strategieën geëvalueerd, namelijk een primaire endovasculaire interventie en primaire bypasschirurgie, bij patiënten zonder voorafgaande vasculaire chirurgische ingrepen aan dezelfde extremiteit. Vroege morbiditeit, maar niet sterfte, was aanzienlijk lager na een primaire endovasculaire interventie in vergelijking met primaire bypasschirurgie, alhoewel dit ten koste ging van meer postoperatieve secundaire revascularisaties. Daarnaast toont de data dat oudere patiënten, arteriële laesies onder de knie en patiënten met meer comorbiditeiten, een grotere kans hebben op het ondergaan van een primaire endovasculaire ingreep. **Hoofdstuk 3** geeft inzicht in het effect dat diabetes mellitus heeft op de lange termijn resultaten van revascularisatie procedures. Insulineafhankelijke diabetes bleek het risico te verhogen op onvolledige wondgenezing, restenose en amputatie in vergelijking met patiënten zonder diabetes, terwijl niet-insulineafhankelijke diabetes niet geassocieerd was met deze lange termijn complicaties. Bovendien suggereert deze studie dat belangrijke complicaties geassocieerd met insulineafhankelijke diabetes mogelijk verminderd kunnen worden in

patiënten die op een zorgvuldige wijze geselecteerd worden voor bypasschirurgie. In **hoofdstuk 4** is de invloed van renine-angiotensine systeem remmers geanalyseerd op de lange termijn uitkomsten van patiënten met kritische ischemie die een revascularisatie ondergaan. Het voorschrijven van een renine-angiotensine systeem remmer bij ontslag was geassocieerd met lagere perioperatieve cardiovasculaire complicaties, evenals een verbeterde 5-jaars overleving van 30%. Het gunstige effect van renine-angiotensine systeem remmers was beperkt tot patiënten die met een hoge dosis van het medicament werden ontslagen. In de **hoofdstukken 5 en 6** worden twee onderbelichte risicofactoren geïntroduceerd bij patiënten met kritische ischemie die bypasschirurgie ondergaan, namelijk preoperatieve anemie en bloedtransfusies. **Hoofdstuk 5** laat zien dat perioperatieve sterfte, amputaties en cardiovasculaire complicaties meer voorkomen bij patiënten met preoperatief afwijkende hematocriet waarden, waarbij de meest ernstig anemische patiënten het hoogste risico hebben op complicaties. Bloedtransfusies, of de vooringenomen perceptie om te transfunderen bij bloedingen, verminderde niet, maar verhoogde juist postoperatieve risico's bij patiënten met milde preoperatieve anemie. In lijn met deze bevindingen is **hoofdstuk 6** toegewijd aan het evalueren van de lange termijn uitkomsten van patiënten met preoperatieve anemie die bypasschirurgie ondergaan. Meer dan de helft van de patiënten met kritische ischemie presenteerde zich met anemie bij opname en zowel het risico op mortaliteit alsook amputatie op lange termijn waren tweemaal hoger voor patiënten met preoperatieve anemie. Bloedtransfusies waren opnieuw sterk gecorreleerd met anemie en het risico op perioperatieve alsmede lange termijn complicaties was hoger na bloedtransfusie in specifieke patiëntengroepen. Uit **hoofdstuk 7** blijkt dat de incidentie van ongeplande heropname binnen 30 dagen na een endovasculaire interventie relatief vaak voorkomt bij kritische ischemie (1 op 6 patiënten) en claudicatio intermittens (1 op de 15 patiënten). Mortaliteit en morbiditeit na de index opname waren significant hoger voor patiënten die opnieuw werden opgenomen in het ziekenhuis. Risicofactoren voor een ongeplande heropname in patiënten met kritische ischemie en claudicatio intermittens omvatten patiëntkenmerken evenals operatieve parameters, maar ook het optreden van complicaties gedurende de index opname, zoals een bloeding en een ongeplande heroperatie. Het succes van een revascularisatie procedure en daarbij de doorbloeding van de onderste extremiteit kan bedreigd worden door het optreden van een restenose of occlusie. **Hoofdstuk 8** laat zien dat eerdere revascularisatie procedures de resultaten van daaropvolgende vasculaire interventies van dezelfde extremiteit wel degelijk beïnvloeden. Bypasschirurgie na eerdere ipsilaterale revascularisatie bleek een onafhankelijke riscofactor te zijn voor amputatie of re-interventie, evenals voor een ongeplande heroperaties binnen 30 dagen. Daarnaast was bypasschirurgie na een eerdere endovasculaire interventie geassocieerd met wondinfecties na de operatie, terwijl meer re-interventies werden uitgevoerd na bypasschirurgie met een voorafgaande bypass procedure.

Restenose oftewel een nieuwe vernauwing in het behandelde vaattraject blijft een belangrijke klinische complicatie na revascularisatie procedures, waarvoor in de meeste

gevallen opnieuw een behandeling noodzakelijk is. *Deel II* had als doel innovatieve en efficiënte strategieën te ontwikkelen met behulp van gentherapie om factoren die bijdragen aan restenose te modificeren en hierbij de lange termijn resultaten van revascularisatie procedures te verbeteren. Met behulp van gentherapie worden genen in de vaatwand gemoduleerd die coderen voor zowel beschermende als schadelijke eiwitten met als gevolg het voorkomen van ongeremde vasculaire remodellering oftewel restenose. In **hoofdstuk 9** wordt een nieuwe transfectiemethode geïntroduceerd in een rattenmodel om het genetische materiaal op de juiste locatie in de vaatwand te krijgen. In een gevalideerd dierenmodel werd effectieve transfectie van cellen aangetoond na lokale intraluminale toediening van het genetisch materiaal gedurende slechts 15 minuten. Deze therapeutische strategie was specifiek gericht op één gen dat nauw betrokken is bij het proces van vasculaire remodellering: trombospondin-2, een matricellulair eiwit. Het uitschakelen van thrombospondin-2 resulteerde in modificatie van de remodellering door remming van belangrijke bijdragende eiwitten, evenals vermindering van cel proliferatie, collageenafzetting en de ontstekingscascade. In lijn met deze resultaten geeft **hoofdstuk 10** inzicht in selectieve uitschakeling van meerdere genen die betrokken zijn bij restenose. Opnieuw werd selectieve uitschakeling toegepast met behulp van non-virale gentherapie specifiek gericht op trombospondin-2 en myristolated alanine-rich C kinase substrate (MARCKS). Langdurige remming van de expressie van bovengenoemde genen werd gevonden tot 21 dagen na lokale transfectie. Het gelijktijdige remmen van twee verschillende pathways betrokken bij restenose verminderde de cellulaire proliferatie nog meer dan de selectieve uitschakeling van deze genen afzonderlijk. De langdurige uitschakeling van deze genen had een verminderde intreding van ontstekingscellen in de vaatwand tot gevolg, samen met een veranderde productie van belangrijke cytokinen en proteïnasen. Deze data bevestigt de potentie van gentherapie, dat niet enkel inzicht geeft in de pathofysiologie van vasculaire remodellering en restenose, maar ook een effectief therapeutisch platform aanreikt om restenose tegen te gaan, evenals de prestaties van revascularisatie procedures substantieel te verbeteren.

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Geachte leden van de leescommissie: prof. dr. R. Goldschmeding (Universiteit Utrecht), prof. dr. R.L.A.W. Bleys (Universiteit Utrecht), prof. dr. D.P.V. de Kleijn (Universiteit Utrecht), prof. dr. G.J. de Borst (Universiteit Utrecht), prof. dr. W. Wisselink (VU Amsterdam) en dr. M.L. Schermerhorn (Harvard University), hartelijk dank voor uw tijd en interesse om zitting te nemen in de leescommissie ter beoordeling van mijn proefschrift.

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CURRICULUM VITAE

Thomas Carel Fredericus Bodewes was born on July 14, 1986 in Baarn, the Netherlands. In 2004, he graduated high school at Het Nieuwe Eemland in Amersfoort and started medical school at the VU Amsterdam. As part of medical school, Thomas gained international working experience through a clerkship surgical oncology in Sydney, Australia. After his graduation, he started working as a non-training resident (ANIOS) at the Department of Surgery at St. Antonius Hospital Nieuwegein, the Netherlands. Thomas started conducting research in vascular surgery evaluating the performance of endovascular repair for type B aortic dissection. Participation in several projects evolved into a PhD program under the supervision of professor Frans L. Moll at the University Medical Center in Utrecht. This brought the opportunity to move to Boston, USA and work on new research projects in basic science together with dr. Frank W. LoGerfo at the Department of Vascular and Endovascular Surgery at Beth Israel Deaconess Medical Center (BIDMC). After one year, Thomas started working with dr. Marc L. Schermerhorn (BIDMC) on several clinical projects related to his thesis subject along with continuing his basic science work. In 2017, after two-and-a-half years in Boston, Thomas moved back to the Netherlands to finish his PhD and the results of his research are presented in this thesis. Thomas will start his surgical residency at the VU Medical Center Amsterdam in July 2017 under the supervision of prof. dr. van der Peet.