CONTEMPORARY OUTCOMES OF DISTAL LOWER EXTREMITY BYPASS FOR CHRONIC LIMB THREATENING ISCHEMIA AND A MODEL BASED COMPARISON WITH NON-SURGICAL THERAPIES

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DEDICATION

This is dedicated to the patients I cared for who had poor outcomes after surgery, prompting me to think critically not just about what we do but also when and why.

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Katherin Eliza Leckie

CONTEMPORARY OUTCOMES OF DISTAL LOWER EXTREMITY BYPASS FOR CHRONIC LIMB THREATENING ISCHEMIA AND A MODEL BASED COMPARISON WITH NON-SURGICAL THERAPIES

Objective: Gold standard therapy for chronic limb threatening ischemia (CLTI) is revascularization but in patients in whom below-the-knee bypass is indicated autologous vein conduit may not be available. Contemporary outcomes of distal bypass with suboptimal conduits have not been well described and recent advances in non-surgical therapies raise the question of whether in some cases there is evidence that these should be considered.

Methods: Data was obtained from the Vascular Quality Initiative (VQI) registry as well as from a multi-center, randomized clinical trial of cell therapy. Incidence of major amputation after distal bypass was estimated for the VQI cohort by conduit type using non-parametric survival analysis with death as a competing risk. A cox proportional hazards model was then fit to the pooled data in a stepwise fashion with death as a competing risk, including evaluations for appropriate transformation, time dependency and interactions for each included covariate, and hazard ratios were estimated for the risk of major amputation by treatment.

Results: At 365 days, the estimated cumulative incidence of major amputation with death as a competing risk is 25% after distal bypass with non-autologous biologic conduit (0.2499, 95% CI 0.2242 - 0.2785), 13% for prosthetic (0.1276, 95% CI 0.1172 - 0.1389) and 9% for GSV (0.0900, 95% CI 0.0848 - 0.0956). The cox proportional hazards model found a significant interaction between age and treatment. Compared to

bypass with non-autogenous biologic, the hazard ratios for bypass with GSV were 0.41 ($p<0.0001$), 0.41 ($p<0.0001$), 0.42 ($p<0.0001$) and 0.42 ($p<0.0001$) respectively at ages 55, 60, 65 and 70 and for bypass with prosthetic were 0.68 ($p=0.0043$), 0.67 ($p=0.0004$), 0.65 ($p<0.0001$) and 0.64 ($p<0.0001$) respectively and for autologous cell therapy 0.22 ($p=0.0005$), 0.34 ($p=0.0011$), 0.52 ($p=0.0196$) and 0.76 ($p=0.3677$) respectively. No significant differences were found between best medical management and distal bypass with non-autologous biologic.

Conclusion: The risk of major amputation after distal bypass is lowest in patients with GSV conduit and highest following bypass with non-autologous biologic. Using a semi-parametric model, cell therapy was estimated to significantly decrease the risk of amputation compared to distal bypass with non-autologous biologic conduit in younger patients.

Giorgos Bakoyannis, Ph.D., Chair

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LIST OF ABBREVIATIONS

Peripheral vascular disease (PVD)

Chronic limb threatening ischemia (CLTI)

Great saphenous vein (GSV)

Polytetrafluoroethylene (PTFE)

Bone marrow derived cells (BMCs)

MarrowStim Treatment of Limb Ischemia in Subjects With Severe Peripheral Arterial Disease (MOBILE) trial

Amputation-free survival (AFS)

The Vascular Quality Initiative (VQI)

Carotid endarterectomy (CEA)

Carotid artery stenting (CAS)

Abdominal aortic aneurysm (AAA)

Endovascular infrarenal AAA repair (EVAR)

Society for Vascular Surgery (SVS)

Patient Safety Organization (PSO)

Below knee amputation (BKA)

Above knee amputation (AKA)

Social Security Death Index (SSDI)

Body mass index (BMI)

Angiotensin-converting enzyme inhibitor (ACE-I)

Chronic obstructive pulmonary disease (COPD)

Coronary artery disease (CAD)

Congestive heart failure (CHF)

Ankle brachial index (ABI)

Toe brachial index (TBI)

One-way analysis of variance (ANOVA)

Akaike information criterion (AIC)

Likelihood ratio (LR)

INTRODUCTION

Peripheral vascular disease (PVD) can manifest as intermittent claudication (IC) or chronic limb threatening ischemia (CLTI). CLTI has a prevalence of 1.3%[1,2] and an estimated incidence of 500-1000/million per year[1] and carries with it a high risk of amputation and mortality even with revascularization[2-5] and higher when there are no revascularization options.[3,6] Restoration of blood flow is the gold standard of treatment, either by open surgical reconstruction or an endovascular intervention. Open reconstruction with autologous great saphenous vein (GSV) has good long term results with 5 year amputation free survival reported >50% and limb salvage rates and approaching 90%.[5,7] Autologous conduit is not always available however and up to 30% of patients who would be candidates for surgical bypass do not have adequate vein.[7,8] Alternative conduits include allografts (cryopreserved arteries, umbilical-cord veins, cryopreserved veins) and prosthetic grafts (polytetrafluoroethylene (PTFE) and polyester (Dacron(R)).

These are all considered inferior to autologous vein in distal bypasses (distal anastomosis below the knee) with less than 25% patency for allografts[9] and less than 50% patency at five years for prosthetic[10,11], although it is suggested the patency of PTFE bypasses may be improved with use of a vein cuff[12] or heparin bonded PTFE.[13,14] Even with improved patency, however, PTFE is inferior to autologous vein with respect to limb salvage, 60% vs 90% at 5 years.[5,7,14,15]

But not all patients undergo reconstruction; between 20% and 40% of new cases of CLTI will have no options or not be candidates for revascularization. The morbidity and mortality of medically managed CLTI is significant but has decreased over the last

couple of decades with estimated one year mortality of 22% and major amputation rate also around 22%.[6,16] This is especially interesting because in a recent retrospective review of open reconstruction with alternative conduits in 240 patients, freedom from major adverse limb events or death at one year was just 60%[17] which is comparable to medically managed CLTI. While non-interventional therapy for CLTI patients primarily consists of optimal medical management of comorbidities and wound care, other potential non-surgical treatments under investigation for no option CLTI patients include intramuscular injections of autologous bone marrow derived cells (BMCs). Since the early 2000s there have been multiple small randomized clinical trials investigating the efficacy of BMCs and meta-analyses provide evidence that cell therapy may decrease major amputation by 40% [18,19].

The MOBILE trial was a multi-center, double-blind, randomized, placebo-controlled trial conducted at 26 centers in the U.S. evaluating the efficacy intramuscular injections autologous bone marrow cells in patient's presenting with Rutherford 4 or 5 critical limb ischemia who had no surgical options or were not considered surgical candidates.[20] 152 patients (155 limbs) were enrolled and randomized 3:1 to concentrated bone marrow nucleated cells (cBMNC) or placebo respectively. Randomization was stratified by investigative site as well as by diabetic status and Rutherford category. The primary endpoint was amputation-free survival (AFS), a composite measure of major amputation of the index limb and all-cause death, at 52 weeks after treatment. The secondary endpoints included major amputation, death, and minor amputation as well as changes from baseline in measures of limb perfusion, ambulation and quality of life. While one year results failed to reach significance, (AFS 80% vs. 70%, p=0.224), the two

year results, as presented at the 2019 Vascular Annual Meeting, were notable for a significant improvement associated with cell therapy with AFS of 77% compared to 56% in the control group (p=0.028). The 70% freedom from events seen in the control group at 1 year again highlights the issue of improved contemporary outcomes in medically managed CLTI and the associated loss of statistical significance in studies powered for a higher baseline event rate.

These promising results of cell-based therapy, combined with the observation that the natural history of untreated CLTI has improved over the last few decades [6] and that outcomes following surgical bypass with alternative conduits are poor [17] raises the question of whether the target population of CLTI patients for whom cell therapy might be considered could and should be expanded to those who are receiving sub-optimal reconstruction. This question is difficult to approach in the existing literature because of the heterogeneity of this patient population and the differing inclusion and exclusion criteria across randomized trials and observational studies (the former being stricter and therefore having healthier patients while the later tend to be cross sections of the population).

The goals of this project was to analyze de-identified patient data from a national prospectively maintained database who had distal bypass to describe contemporary outcomes of distal bypass with alternative conduit. Next, a pooled dataset including the de-identified patient data from the MOBILE trial was used to compare outcomes following cell therapy or medical management only with the surgical reconstruction cohort by fitting an appropriate semi-parametric survival model to adjust for differences in baseline demographics and risk factors.

METHODS

Study Design

The Vascular Quality Initiative (VQI) collects demographic, clinical, procedural and outcomes data on selected commonly performed vascular procedures in participating institutions across the USA and Canada. These procedures include carotid endarterectomy (CEA), carotid artery stenting (CAS), infrainguinal and suprainguinal bypass, open infrarenal abdominal aortic aneurysm (AAA) repair, endovascular infrarenal AAA repair (EVAR), thoracic endovascular aortic repair, including branch and fenestrated AAA repair, peripheral vascular intervention of aortoiliac and lower extremity arterial disease, and hemodialysis access. The selection of the variables collected at each site and for each procedure was motivated by the goal of ensuring enough patient information and procedural details for risk-adjustment and process analysis as well as key outcome information. Participating locations agree to collect all the specified variables as well as contact the patients for at least a 1 year follow up. The primary purpose of the VQI is to provide data and analyses to the participating centers for internal quality initiatives in accordance to the Patient Safety Act. But aggregate de-identified data is also made available for research projects as approved the Society for Vascular Surgery (SVS) Patient Safety Organization (PSO) Quality Committee.[21]

For this analysis, data from the Infra-Inguinal Bypass registry was requested and obtained from the SVS PSO. This dataset included patients who had a lower extremity bypass between 2009 and 2019. Procedures are assigned unique ID numbers upon entry into the database and this was used to unite de-identified follow up data with the

procedural and demographic information obtained from the index procedure entry. Unique center IDs were used for clustering.

Patients with non-missing amputation status at any follow up time after their primary procedure were isolated. Major amputation was defined as either below knee (BKA) or above knee (AKA) or unspecified major amputation (retired terminology). Time of event was recorded as days between index procedure and major amputation. Patients who never underwent an amputation were censored on the last recorded long-term follow up contact at which amputation status was assessed or on death if this occurred before or within 30 days of last recorded long-term follow up contact. If the patient died within this follow up window, death was coded as a competing event. In those cases where survival was assessed using the Social Security Death Index (SSDI) death might be recorded significantly later than the time of last contact with the patient. So in the case of death greater than 30 days after the last recorded follow up then they were censored at the time of contact. For example, a patient who was contacted at 202 days after surgery and had not undergone amputation at that time but is noted to have died 372 days after their index surgery is censored without event at 202 days because their amputation status in the intervening 170 days is unknown. Additionally, as all participating centers attempt to obtain follow up data on patients in the registry within a 9-21 month window from the index procedure to comply with the 1 year follow up requirement but only some continue to collect patient data over a longer timeframe, for the purposes of this analysis all patients are censored after the common follow up period at 640 days or approximately 21 months.

The patient population of interest was then identified as those with a below knee bypass, defined as a recipient vessel at or distal to the below knee popliteal artery but proximal to the foot (i.e. tarsal or plantar bypasses were excluded), with any of GSV, prosthetic or biologic (non-autologous artery or vein) grafts. Non-GSV autologous vein grafts and composite grafts were excluded. Patients with bypass for aneurysmal pathology or acute ischemia were excluded.

Potential predictor variables included demographics as well as risk factors and comorbidities. The included age at time of surgery in years, gender as birth sex, race as a factor consisting of black, white (non-Hispanic) and other and body mass index (BMI). Medication use history included pre-operative use of aspirin, $P2Y_{12}$-receptor blocker, angiotensin-converting enzyme inhibitor (ACE-I), beta-blocker, statin or anticoagulant. Medical history included smoking status as current, prior or never, diabetes as none, diet-controlled/oral agents only or insulin dependent and chronic obstructive pulmonary disease (COPD) as none, medication controlled or oxygen dependent. Coronary artery disease (CAD), congestive heart failure (CHF), dialysis, prior bypass and prior percutaneous interventions were included present or not. Perioperative factors included ischemia as no chronic-limb-threatening ischemia, rest pain or tissue loss, ambulatory status as either ambulatory (with or without assistance) or non-ambulatory (wheelchair or bedridden), preoperative serum creatinine, hemoglobin, ankle brachial index (ABI) and toe brachial index (TBI).

Statistical Analysis of VQI data

The first aim of this analysis was to evaluate contemporary outcomes of distal bypass by conduit. A descriptive comparison of the potential predictor variables was

made across the three types of conduit, biologic (non-autologous), prosthetic and GSV and means or percentages were reported as appropriate. One-way analysis of variance (ANOVA) was used for continuous variables and chi-square tests for factors.

Non-parametric estimates of the probability of major amputation, the outcome of interest, were then obtained. Given that patients may die without having experienced an amputation, death was treated as a competing risk for this analysis and the population-averaged cumulative incidence function was estimated using the nonparametric working-independence version of the Aalen-Johansen estimator.[22] Cumulative incidence estimates were stratified by conduit across the entire cohort. Center ID was used to group observations for robust variance estimation using an infinitesimal jackknife approach.

Additional stratification by select potential predictors of major amputations is serially performed for the cohort that received a distal bypass with prosthetic conduit as this patient group may represent a point of clinical equipoise for many practitioners. Predictors considered include diabetes, smoking status, race and degree of ischemia on presentation, target vessel, end-stage renal disease, ambulatory status and history of lower extremity bypass. Pairwise equality between groups within each stratum was tested as proposed by Gray (1988).[23] The Bonferroni method was used to correct for multiple comparisons. Probability of amputation with death as a competing risk was estimated at 1 year for all strata. Confidence intervals calculated using robust variance estimates to account for the within-center dependence.

Statistical Analysis of pooled data

The second aim of this analysis was to compare outcomes of distal bypass with any of the three conduit types with best medical management or autologous cell therapy

and to this end a subset of the VQI data was pooled with de-identified data from the MOBILE trial. Patients in the VQI dataset with severe congestive heart failure or on dialysis were not included as these were exclusion criteria for the MOBILE trial. A comparison of potential predictor variables was made between the two patient sources and means or percentages were reported as appropriate. T-tests were used for continuous variables and chi-square tests for factors.

Cause-specific hazard of major amputation was then modeled using a semiparametric Cox proportional hazard model and censoring death. Center ID was used to group observations for robust variance estimation using an infinitesimal jackknife approach in order to account for the within-center dependence. A base model was fit using treatment (five levels for bypass with GSV, prosthetic or non-autogenous biologic, medical management only or cell therapy). Candidate predictors (listed previously) were screened for inclusion in further model selection by conducting a likelihood-ratio chi-square test for the addition of each to the base model. A significance level threshold of 0.2 was used. Variables with greater than 10% missing values were excluded. Logarithmic and exponential transformations of continuous variables were considered at this stage as candidates for inclusion.

Identified predictors were then included in a stepwise model selection procedure using Akaike information criterion (AIC). Type III analysis of variance tests (likelihood ratio (LR)) are conducted on the variables included in the last iteration and only those with p-value less than 0.05 are left in the model.

The resulting model was evaluated for violations of the proportional hazards assumption by plotting the scaled Schoenfeld residuals against transformed time and

testing for correlation.[24] Variables with indication of correlation (p-value < 0.05) were evaluated for a significant time interaction. This was fit by splitting each patient observation into 30 day intervals and adding an interaction term between the variable violating the proportional hazards assumption and the start time of the interval. Both linear, logarithmic and exponential transformations of start time were considered. Additionally, a model was considered that allowed piecewise proportional hazards with constant coefficients between 0 and 90 days (early), 90 and 365 days (medium) and after 365 days (late). Models were compared by AIC.

Type II analysis of variance tests (Wald) are conducted on time interactions and only those with p-value less than 0.05 remain in the model. Two-way interactions between all main effects included in the model at this stage were then screened for inclusion in further model selection by conducting a likelihood-ratio chi-square test for the addition of each to the base model. A significance level threshold of 0.2 was used to select interaction terms to include in a forwards selection procedure using AIC. Type II analysis of variance tests (Wald) are conducted on the interaction terms included in the last iteration and only those with p-value less than 0.05 remain in the final model.

A forest plot for the hazard ratios with 95% confidence intervals associated with the levels of treatment is generated for this final model with Biologic graft as the reference level. Similar representations of the hazard ratios associated with diabetes, smoking status, race and degree of ischemia on presentation are also constructed. In the case of time dependency, the hazard ratios are calculated for 1 year. In the case of any other interaction, the hazard ratios at specified levels of the interacting covariate(s) are reported.

RESULTS

Patient selection

Patient entries from the VQI Infra-Inguinal Bypass registry were united using the unique primary procedure identification number. 43,168 unique procedures were identified and those with missing conduit type, indication for surgery or amputation status on follow up were excluded. 31,591 (73.2%) were found with this minimum complete follow up and this cohort was then narrowed to those with below the knee bypass with either GSV, prosthetic or non-autologous biologic conduits. Composite and non-GSV autologous vein grafts, tarsal and pedal bypass targets and procedures performed for aneurysmal disease or acute ischemia were excluded. This final distal bypass cohort including 17,111 unique procedures. (Figure 1)

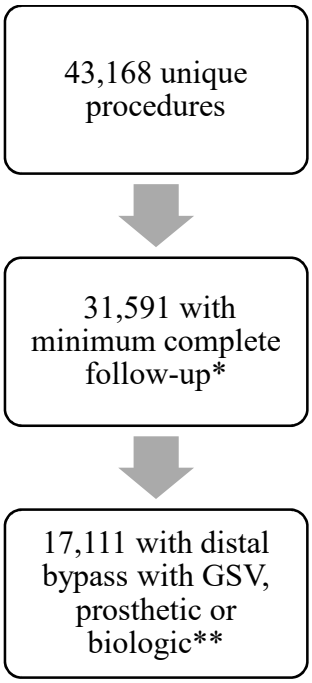

Figure 1: Diagram of patient selection from VQI Infra-Inguinal Bypass registry.
**excluding patients with missing indication for surgery, conduit type or amputation status*
***excluding acute ischemia, aneurysmal pathology and tarsal or plantar bypasses*

Descriptive statistics and non-parametric survival analysis in the VQI cohort

A comparison of the potential predictor variables was made across the three types of conduit: biologic (non-autologous), GSV and prosthetic. These are presented in Table 1. The percent missing entries for each covariate is reported as well as the p-value for a chi-square test or ANOVA for the categorical and continuous predictors respectively.

Categorical Covariate	**Biologic: percent (n)**	**Prosthetic: percent (n)**	**GSV: percent (n)**	**Percent Missing Data**	**P-value: chi-square**
Gender				0.02%	< 0.0001
Female	38.01% (420)	37.4% (1489)	29.6% (3558)		
Preop Smoking				0.06%	< 0.0001
Never	19.4% (214)	13.14% (523)	17.57% (2111)		
Prior	52.22% (576)	50.46% (2008)	42.45% (5102)		
Current	28.38% (313)	36.39% (1448)	39.98% (4805)		
HTN				0.01%	< 0.0001
Yes	92.04% (1017)	91.59% (3647)	87.79% (10555)		
Prior bypass				0.01%	< 0.0001
Yes	60.27% (666)	44.53% (1773)	24.51% (2946)		
Prior percutaneous intervention				0.05%	< 0.0001
Yes	54.31% (599)	52.89% (2105)	42.15% (5067)		
CAD				0.05%	< 0.0001
Yes	41.67% (460)	34.82% (1386)	28.73% (3453)		
COPD				0.05%	< 0.0001
No	70.83% (782)	72.59% (2890)	76.88% (9239)		
Yes, medication only	26.9% (297)	24.79% (987)	21.52% (2586)		
Yes, on oxygen	2.26% (25)	2.61% (104)	1.61% (193)		

Preop aspirin				0.04%	0.3948
Yes	73.46% (811)	74.16% (2950)	73.06% (8783)		
Preop $P2Y_{12}$ inhibitor				0.08%	< 0.0001
Yes	37.35% (412)	36.11% (1436)	28.37% (3409)		
Preop statin				0.04%	< 0.0001
Yes	79.26% (875)	76.65% (3050)	71.49% (8594)		
Preop beta blocker				0.11%	< 0.0001
Yes	66.61% (736)	63.4% (2520)	58.86% (7071)		
Preop ACE-I				19.03%	< 0.0001
Yes	55.21% (519)	56.06% (1930)	51.66% (4893)		
Preop anticoagulation				19.06%	< 0.0001
Yes	28.24% (266)	19.7% (678)	16.32% (1545)		
Race				0.01%	< 0.0001
Black	16.92% (187)	18.56% (739)	15.07% (1812)		
White	75.57% (835)	77.24% (3075)	80.75% (9709)		
Other	7.51% (83)	4.19% (167)	4.18% (502)		
Ambulatory				0.32%	< 0.0001
Yes	90.03% (993)	95.19% (3779)	94.96% (11379)		
CHF				0.02%	< 0.0001
Yes	25.79% (285)	17.78% (708)	15.38% (1849)		
ESRD				0.01%	< 0.0001
Yes	10.14% (112)	4.87% (194)	5.43% (653)		
Diabetes				0.01%	< 0.0001
No	41.39% (457)	48.92% (1948)	46.62% (5606)		
Yes, diet or oral medications	23.19% (256)	23.46% (934)	23.31% (2803)		
Yes, on insulin	35.42% (391)	27.62% (1100)	30.06% (3615)		

Target				0%	< 0.0001
Below Knee Popliteal	17.47% (193)	63.36% (2523)	41.34% (4971)		
Tibioperoneal Trunk	3.35% (37)	6.35% (253)	6.1% (733)		
Tibial Vessels	79.19% (875)	30.29% (1206)	52.56% (6320)		
Ischemia				0%	< 0.0001
No CLTI	10.14% (112)	27.17% (1082)	24.14% (2902)		
Rest Pain	25.97% (287)	32.62% (1299)	26.35% (3168)		
Tissue Loss	63.89% (706)	40.21% (1601)	49.52% (5954)		
Elective				0.02%	< 0.0001
Yes	75.63% (835)	81.19% (3232)	82.17% (9878)		
Continuous Covariate	**Biologic: mean (n)**	**Prosthetic: mean (n)**	**GSV: mean (n)**	**Missing Entries**	**P-value: ANOVA**
Age (years)	69.3 (1105)	68.23 (3980)	66.18 (12022)	0.04%	< 0.0001
Creatinine (mg/dl)	1.11 (983)	1.07 (3760)	1.09 (11312)	6.18%	0.134
Hemoglobin (g/dl)	11.48 (1059)	12.11 (3828)	12.42 (11160)	6.23%	< 0.0001
Pre-op ABI	0.56 (726)	0.52 (2994)	0.58 (9106)	25.05%	< 0.0001
Pre-op TBI	0.22 (340)	0.23 (1196)	0.25 (3644)	69.73%	0.1301
BMI	26.95 (1103)	27.07 (3959)	27.89 (11961)	0.53%	< 0.0001

Table 1: Distribution of potential predictor variables by conduit type. Reported p-values are for a chi-square test or ANOVA for the categorical and continuous predictors respectively

As we would suspect, there are multiple significant differences in the distribution of demographic, medical and perioperative factors between the three conduit types. In general, patients who had a bypass with GSV appear to be younger, more likely male, more likely to be currently smoking but with fewer comorbidities and prior procedures. Among the evaluated comorbidities, only in end-stage renal disease (ESRD) and insulin

dependent diabetes (IDDM) is there a lower percentage of patients in the cohort that underwent prosthetic bypass compared to the GSV cohort.

Because of this unbalanced distribution of potential risk factors, a comparison in outcomes between conduit types will necessarily reflect the contribution of those risk factors as well as the graft type. Such a comparison is still of value however, as this national, prospectively-collected database likely reflects actual practice patterns.

To obtain non-parametric estimates of the probability of major amputation, death was treated as a competing risk and marginal probability estimates using a cumulative incidence function were employed. Cumulative incidence estimates were stratified by conduit across the entire cohort. (Figure 2)

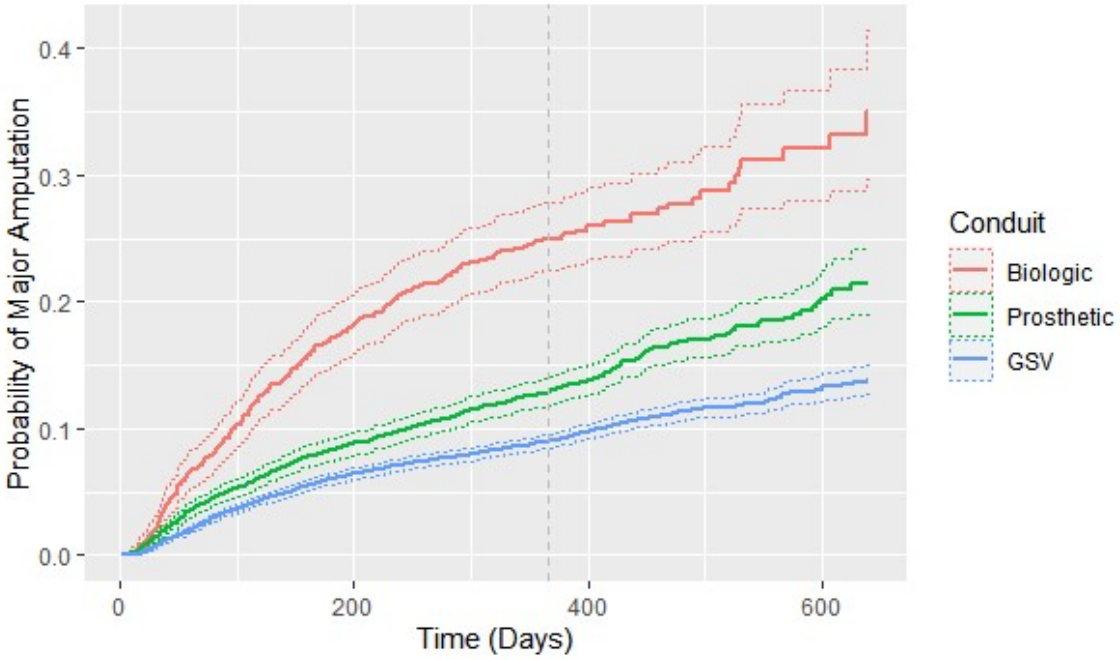

Figure 2: Population-averaged cumulative incidence of major amputation after distal bypass by conduit. Dashed vertical line at 365 days.

Pairwise, these curves are statistically significantly different by Gray's test with a p-values < 0.0001. At 365 days, the estimated population-averaged probability of major

amputation with death as a competing risk is 25% after distal bypass with non-autologous biologic conduit (0.2499, 95% CI 0.2242 - 0.2785), 13% for prosthetic (0.1276, 95% CI 0.1172 - 0.1389) and 9% for GSV (0.0900, 95% CI 0.0848 - 0.0956).

Additional stratification by potential predictors of major amputations is performed for the 3,982 patients that received a distal bypass with prosthetic conduit as this patient group may represent a point of clinical equipoise for many practitioners.

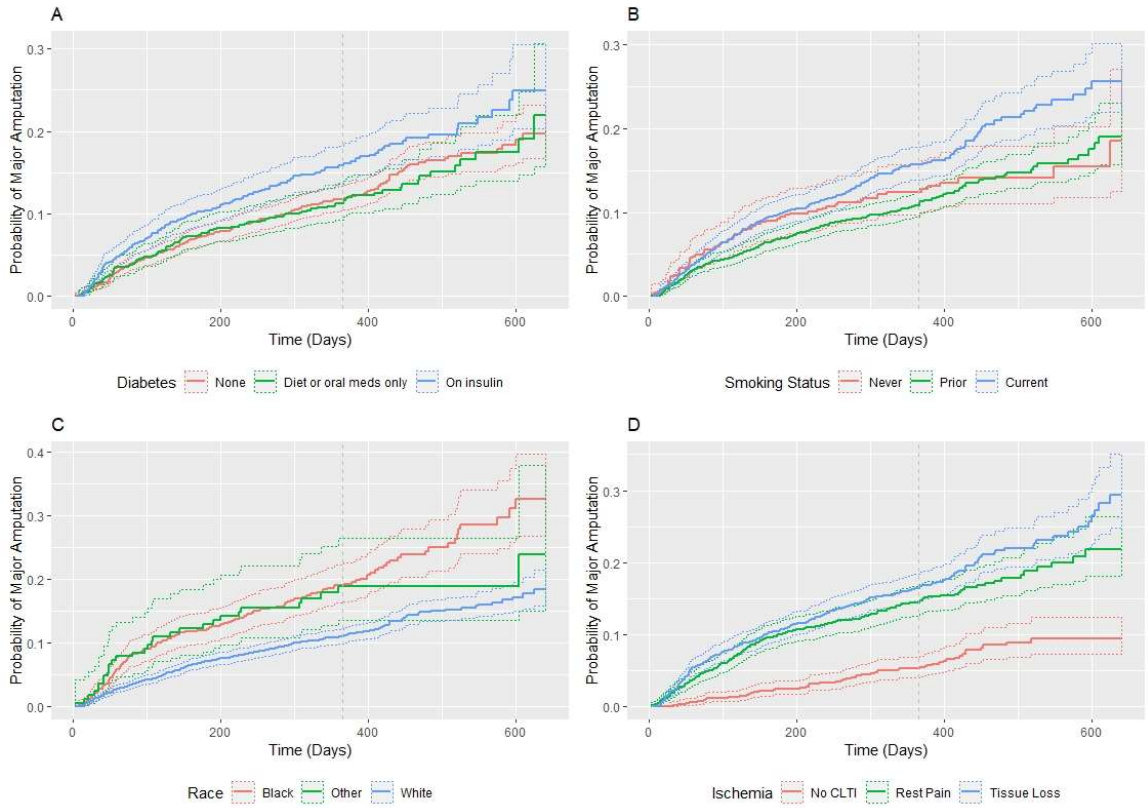

Figure 3: Population-averaged cumulative incidence of major amputation after distal bypass with prosthetic graft by covariates - part 1. Stratified by diabetes (A), smoking status (B), race (C) and degree of ischemia (D). Dashed vertical line at 365 days.

In figure 3, the cumulative incidence of major amputation after distal bypass with prosthetic conduit is plotted by diabetic status, smoking status, race and degree of ischemia. At 365 days, the estimated marginal probability of major amputation with death as a competing risk is 12% for non-diabetics (0.1178, 95% CI 0.1037 - 0.1339), 11% in

those with diet-controlled diabetes or on oral medications only (0.1123, 95% CI 0.0931 - 0.1356) and 16% for patients on insulin (0.1579, 95% CI 0.1369 - 0.1823).

The corrected α-level for 3 pairwise comparisons using the Bonferroni method is 0.0167.

Pairwise by Gray's test, the insulin-dependent diabetics are significantly different than both the non-diabetics ($p = 0.0025 < 0.0167$) and the diabetics on diet or oral therapy ($p = 0.0049 < 0.0167$) but there is no significant difference between the non-diabetic and diet/oral medication controlled cohorts ($p = 0.7270 > 0.0167$).

By smoking status at time of the index surgery, the estimated probability of major amputation at 1 year is 12% for never-smokers (0.1237, 95% CI 0.0975 - 0.1569), 11% for previous smokers (0.108, 95% CI 0.0947 - 0.1232) and 16% for current smokers (0.1566, 95% CI 0.1381 - 0.1775). Pairwise by Gray's test, the current smokers are significantly different compared the prior smokers ($p < 0.0001 < 0.0167$) but there is no significant difference between current and never smokers using the Bonferroni corrected α-level ($p = 0.0394 > 0.0167$) or between the prior and never smokers ($p = 0.4956 > 0.0167$).

Stratified by race, the risk of major amputation is estimated at 19% in black patients (0.1926, 95% CI 0.1647 - 0.2253), 11% in whites (0.11, 95% CI 0.099 - 0.1222) and 19% in other races (0.1897, 95% CI 0.1358 - 0.2648). Pairwise by Gray's test, the white cohort is significantly different than both the black cohort ($p < 0.0001 < 0.0167$) and the other cohort ($p = 0.0114 < 0.0167$) but there is no significant difference between the black cohort and other races ($p = 0.0955 > 0.0167$).

By ischemic status of the index limb at time of surgery, the probability of amputation at 1 year is 5% for those without CLTI (0.0525, 95% CI 0.0403 - 0.0685), 14% in those with rest pain (0.1449, 95% CI 0.1262 - 0.1664) and 16% if the patient presented with tissue loss (0.1645, 95% CI 0.1465 - 0.1847). Pairwise by Gray's test, the cohort without CLTI is significantly different than both the rest pain ($p < 0.0001 < 0.0167$) and the tissue loss groups ($p < 0.0001 < 0.0167$) but the separation between the rest pain and tissue loss cohorts did not reach significance ($p = 0.0529 > 0.0167$).

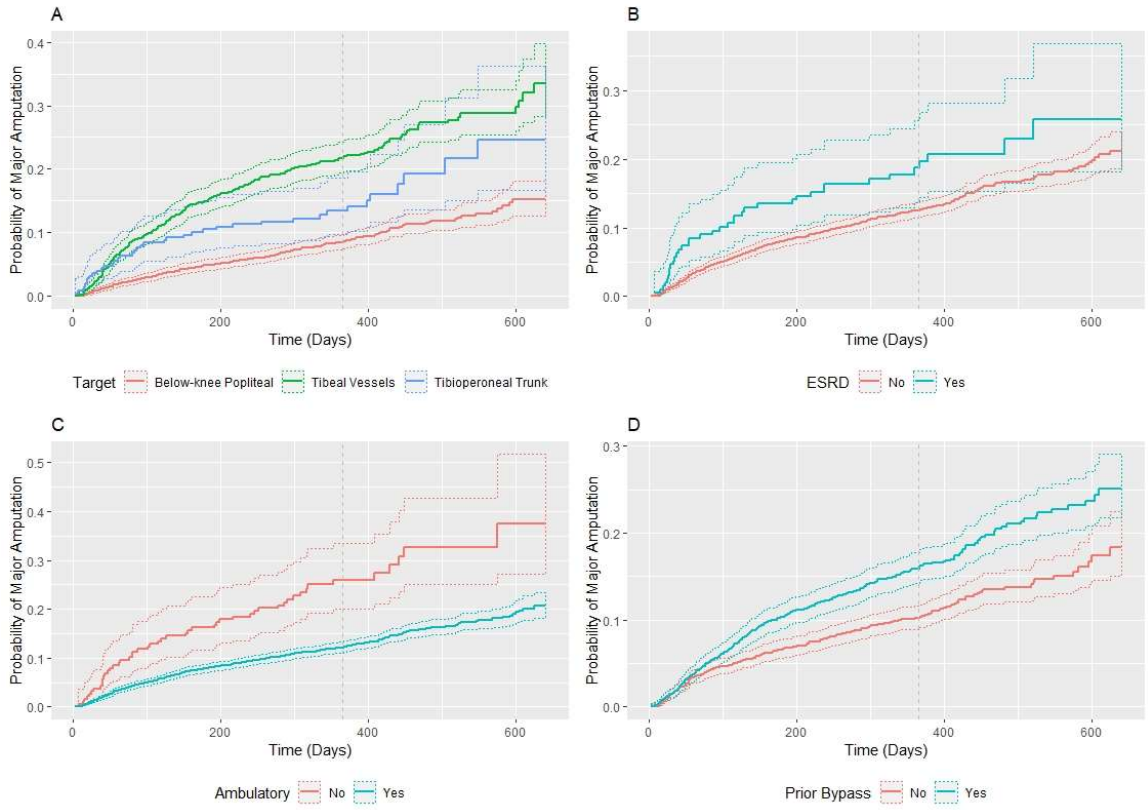

Figure 4: Population-averaged cumulative incidence of major amputation after distal bypass with prosthetic graft by covariates - part 2. Stratified by target vessel (A), end-stage renal disease (B), ambulatory status (C) and history of lower extremity bypass (D). Dashed vertical line at 365 days.

In figure 4, the cumulative incidence of major amputation after distal bypass with prosthetic conduit is plotted by target outflow vessel, end-stage renal disease, ambulatory

status and history of lower extremity bypass. At 365 days, the estimated marginal probability of major amputation is 8% if the outflow was the below-knee popliteal artery (0.0842, 95% CI 0.0735 - 0.0965), 13% if the target was the tibioperoneal trunk (0.1341, 95% CI 0.0968 - 0.1858) and 22% if the outflow was one of the tibial vessels (0.2169, 95% CI 0.1939 - 0.2425). Pairwise by Gray's test, the below-knee popliteal target is significantly different from both the bypasses targeted to the tibioperoneal trunk ($p = 0.0015 < 0.0167$) or the tibial vessels ($p < 0.0001 < 0.0167$) and there is also a significant difference between the tibioperoneal trunk and the tibial vessels as outflow target ($p = 0.0106 < 0.0167$).

For the final three strata, there are just two groups being compared so the Bonferroni correction was no longer necessary. For patients on dialysis the likelihood of major amputation at 1 year is 19% (0.1872, 95% CI 0.1369 - 0.256) compared to 12% in the absence of renal failure (0.1246, 95% CI 0.1141 - 0.1361) and this difference is statistically significant with a p-value of 0.0111. Stratified by ambulatory status prior to index surgery, the risk of major amputation is estimated at 12% if the patients is ambulatory (0.1209, 95% CI 0.1105 - 0.1322) and 26% if the patient is non-ambulatory (0.2594, 95% CI 0.2006 - 0.3353) and these are groups are significantly different with a p-value < 0.0001. In patients with a history of lower extremity bypass, the probability of amputation at 1 year is 16% (0.1594, 95% CI 0.1425 - 0.1783) compared to 10% in those without prior bypass (0.1018, 95% CI 0.0895 - 0.1159). These groups are significantly different with a p-value of < 0.0001.

Overall, these non-parametric estimations of incidence of major amputation in the prosthetic cohort reveal expected associations between known risk factors and

amputation, including worse outcomes with insulin dependent diabetes, current smoking status, non-white race, presence of CLTI, ESRD and non-ambulatory status and prior revascularization surgery. Interestingly however, there is no significant difference between non-diabetics and those with diet/oral medication controlled diabetes. Also, there appears to be an increased early risk in non-smokers which is very similar to current smokers but the curves separate after ~150 days so that ultimately the 1 year risk in non-smokers is more similar prior smokers. While this observation invites speculation, in this non-randomized patient cohort there is expected to be unequal distribution of other risk factors across the strata so interpretation should be undertaken with care.

Descriptive statistics and semi-parametric survival analysis in the pooled cohort

Next a subset of the VQI data was pooled with de-identified data from the MOBILE trial and a comparison of the potential predictor variables was made across the two patient sources: VQI and MOBILE. These are presented in Table 2. The percent missing entries for each covariate is reported as well as the p-value for a chi-square test or t-test for the categorical and continuous predictors respectively.

Covariate	**MOBILE: percent (n)**	**VQI: percent (n)**	**Percent Missing Data**	**P-value**
Gender			0.02%	0.016
Female	41.29% (64)	31.89% (5120)		
Smoking status			0.06%	< 0.0001
Never	21.29% (33)	15.76% (2529)		
Prior	61.94% (96)	44.74% (7179)		
Current	16.77% (26)	39.5% (6339)		
HTN			0%	1
Yes	88.39% (137)	88.51% (14211)		
Prior Bypass			0.01%	< 0.0001
Yes	68.39% (106)	31.84% (5111)		
Prior percutaneous intervention			0.05%	< 0.0001

Yes	70.97% (110)	45.55% (7310)		
CAD			0.05%	0.7518
Yes	31.61% (49)	30.12% (4833)		
COPD			0.04%	0.4708
No	73.55% (114)	75.44% (12108)		
Yes, on medications	25.81% (40)	22.82% (3663)		
Yes, on oxygen	0.65% (1)	1.73% (278)		
Preop aspirin			0.04%	0.2542
Yes	69.03% (107)	73.43% (11784)		
Preop P2Y inhibitor			0.08%	0.0569
Yes	38.06% (59)	30.65% (4917)		
Preop statin			0.04%	0.0201
Yes	81.94% (127)	73.32% (11767)		
Preop beta blocker			0.1%	0.9369
Yes	58.71% (91)	59.35% (9519)		
Preop ACE-I			18.59%	0.5179
Yes	50.97% (79)	53.9% (7030)		
Preop Anticoagulation			18.63%	< 0.0001
Yes	48.39% (75)	17.93% (2337)		
Race			0.01%	0.3115
Black	18.06% (28)	15% (2409)		
White	76.13% (118)	80.81% (12974)		
Other	5.81% (9)	4.19% (672)		
Ambulatory			0.33%	< 0.0001
Yes	84.67% (127)	95.28% (15252)		
CHF			0%	0.3268
Yes	11.61% (18)	14.74% (2367)		
Diabetes			0.01%	0.0202
No	59.35% (92)	48.62% (7806)		
Yes (diet or oral meds)	16.13% (25)	23.48% (3770)		
Yes (on insulin)	24.52% (38)	27.9% (4479)		
Ischemia			0%	< 0.0001
No CLTI	0% (0)	25.19% (4045)		
Rest Pain	56.77% (88)	28.62% (4596)		
Tissue Loss	43.23% (67)	46.18% (7415)		

Elective			0.02%	< 0.0001
Yes	100% (155)	81.95% (13155)		
Covariate	**MOBILE: mean value (n)**	**VQI: mean value (n)**	**Number of Missing Entries**	**P-value**
Age (years)	64.93 (155)	66.94 (16050)	0.04%	0.02105
Creatinine (mg/dl)	1.05 (155)	1.09 (15958)	0.6%	0.18066
Hemoglobin (g/dl)	12.91 (155)	12.41 (15080)	6.02%	0.0004
Pre-op ABI	0.47 (136)	0.55 (12140)	24.27%	0.00394
Pre-op TBI	0.19 (89)	0.25 (4801)	69.84%	0.00358
BMI	28.31 (154)	27.64 (15975)	0.51%	0.16137

Table 2: Distribution of potential predictor variables by patient source. Reported p-values are for a chi-square or t-test for the categorical and continuous predictors respectively.

Unsurprisingly, there are multiple significant differences in the distribution of demographic, medical and perioperative factors between the VQI and MOBILE data. Notably, patients who participated in the clinical trial had a significantly higher incidence of previous bypass and percutaneous interventions. The mean pre-procedural ABI or TBI was also lower in the MOBILE patients and they were less likely to be current smokers or diabetic and more likely to be female or on anticoagulation.

This unbalanced distribution of potential risk factors cannot but highlight that even with an appropriate model to control for significant predictors of major amputation in the absence of randomization it is impossible to guarantee that all biases have been accounted for. Nevertheless, a model still provides a mechanism to control for the effects of many covariates and thus will allow an initial comparison of outcomes of distal bypass in the VQI data set with best medical management or autologous cell therapy in the MOBILE data set. For this purpose, cause-specific hazard of major amputation was

estimated from the pooled data using a Cox proportional hazard model and right-censoring death.

Model fitting was performed as described above. Preoperative ACE-I and anticoagulation, ABI and TBI were excluded for missing greater than 10% of entries. Categorical main effects included in the final model were treatment, degree of ischemia on presentation, history of lower extremity bypass, ambulatory status prior to index procedure, whether the procedure was elective, diabetic status, preoperative smoking status, diagnosis of CHF and preoperative aspirin use. Continuous main effects included BMI and log transformations of age, preoperative hemoglobin and preoperative serum creatinine respectively. A significant time interaction (i.e. correction for violation of proportional hazards assumption) was found for preoperative smoking status, preoperative aspirin use, degree of ischemia on presentation, whether the index procedure was elective and for BMI.

This time dependence was modeled by splitting each patient observation into 30 day intervals and fitting an interaction between the main effect and a log transformation of the start time of each interval. This log transformation resulted in the best fit by AIC compared to linear or exponential transformation and compared to the piecewise proportional hazards model. The final model had no significant violation of proportional hazards with all p-values > 0.05. A number of significant interactions between main effects were found and are listed in table 3.

Main effect:	**Interaction with:**	**P-value**
Treatment	Log Age	=0.0407
Ischemia	Log Time	=0.0006
	Elective	=0.0019
Race	Ambulatory	=0.0013
Log Age	Treatment	=0.0407

	Smoking	=0.0234
	CHF	=0.0312
Log Hemoglobin	Prior Bypass	=0.0332
Prior Bypass	BMI	=0.0028
	Log Hemoglobin	=0.0332
Ambulatory	Race	=0.0013
Elective	Log Time	=0.0047
	Ischemia	=0.0019
	Diabetes	=0.0118
BMI	Log Time	=0.0132
	Prior Bypass	=0.0028
Diabetes	Smoking	=0.0009
	Elective	=0.0118
Log Creatinine		
Smoking	Log Time	=0.0213
	Diabetes	=0.0009
	Log Age	=0.0234
CHF	Log Age	=0.0312
Preop Aspirin	Log Time	=0.0079

Table 3: Main effects included in the final model with their respective significant interactions. P-value is provided for the Type II chi-square test (Wald) for inclusion of interaction term in the model.

In the presence of significant interactions, main effects can only be interpreted in the context of those interaction. Treatment was found to have a significant interaction with log transformed age. In figure 5, the hazard ratios of major amputation for each treatment group are estimated at ages 55, 60, 65 and 70 with non-autologous biologic conduit as the reference level. (Bypass with non-autologous biologic conduit was selected as the reference level rather than best medical management as the small sample size in the latter group from the MOBILE trial and the associated large standard error resulted in few meaningful comparisons.) Both distal bypass with GSV and bypass with prosthetic significantly decrease the risk of major amputation across all ages compared to bypass with non-autologous biologic conduit (Figure 5). Estimated hazard ratios for bypass with GSV at ages 55, 60, 65 and 70 compared with bypass with biologic were 0.41 (95% CI

0.32 - 0.52), 0.41 (95% 0.33 - 0.51), 0.42 (95% 0.34 - 0.51) and 0.42 (95% 0.34 - 0.52) respectively and analogously for bypass with prosthetic were 0.68 (95% CI 0.53 - 0.89), 0.67 (95% CI 0.53 - 0.83), 0.65 (95% CI 0.53 - 0.80) and 0.64 (95% CI 0.52 - 0.79) respectively. GSV is also superior to prosthetic across all ages (hazard ratios of 0.60, 0.62, 0.64 and 0.66 respectively, p-values < 0.0001, see Appendix A for figure). These show little evidence of significant interaction with age however this is notable in the change in the hazard ratio of major amputation for the MarrowStim (autologous cell therapy) group with increasing age. In younger patients there is a significantly decreased risk of amputation in the cell therapy group compared to bypass with non-autologous biologics with estimated hazard ratios of major amputation of 0.22 (95% CI 0.09 - 0.51), 0.34 (95% CI 0.18 - 0.65) and 0.52 (95% CI 0.30 - 0.90) at ages 55, 60 and 65 respectively (Figure 5) and at 55 and 60 years this decreased risk is also significant compared to bypass with prosthetic conduit with hazard ratios of major amputation of 0.32 (95% CI 0.14 - 0.73) and 0.51 (95% CI 0.28 - 0.95) respectively (see Appendix A for figure). By age 70 however, the cell therapy group is no longer significantly different than biologic bypass with a hazard ratio of 0.76 (0.42 - 1.38).

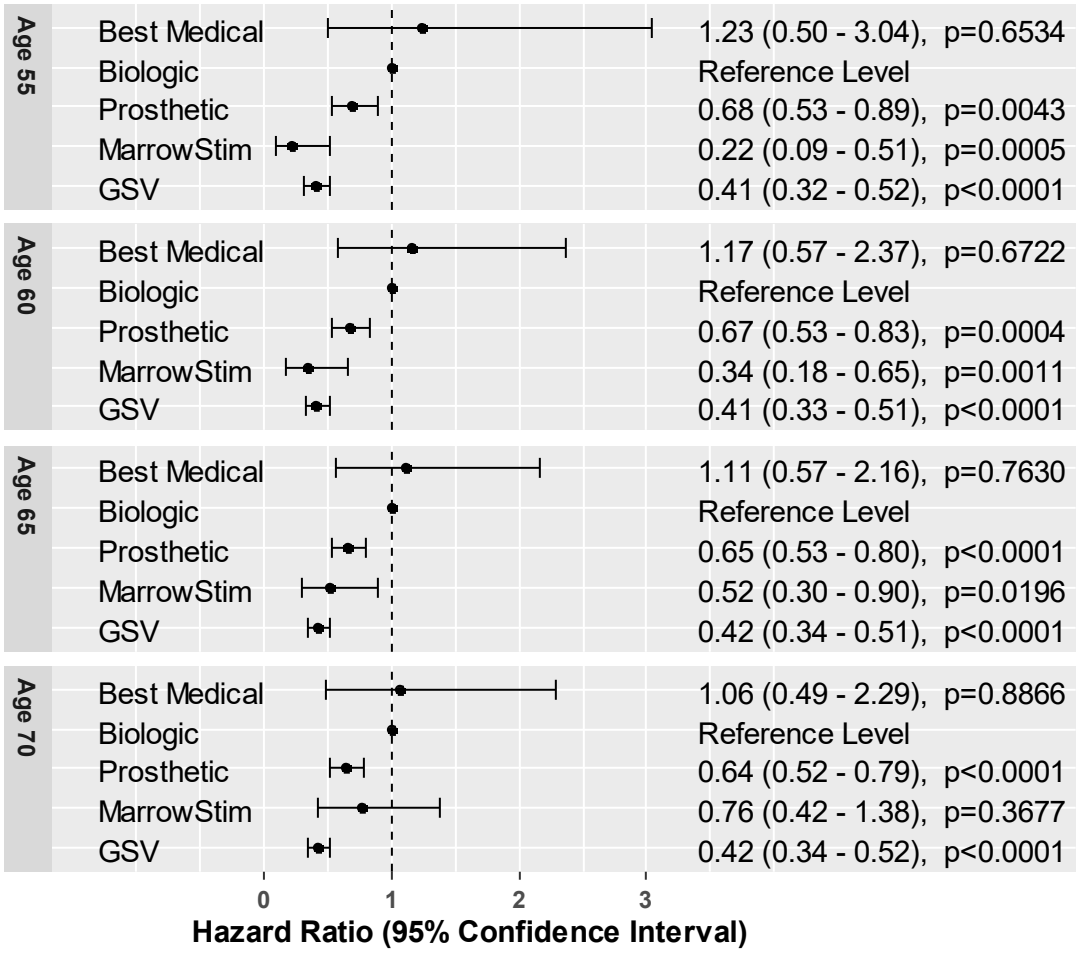

Figure 5: Hazard ratio of major amputation and 95% confidence interval for each treatment group compared to biologic bypass and adjusted for age.

Analogously, hazard ratios for major amputation were calculated for diabetes, smoking status, race and degree of ischemia and these estimates were stratified by the respective covariates with which there were significant interactions. Diabetes was found to have a significant interaction with preoperative smoking status and whether the procedure was elective. Hazard ratios for major amputation by diagnosis of diabetes were stratified by smoking history and were estimated for elective patients (elective rather than urgent/emergent as the former represented 82% of the cohort) and are presented in figure 6. In never smokers, a diagnosis of either level of diabetes is associated with an increased risk of major amputation; hazard ratio of 1.92 (95% CI 1.43 - 2.58) in insulin-dependent

diabetes and 1.53 (95% CI 1.07 - 2.18) in diabetics on diet or oral medications compared to non-diabetic patients. However, in those patients who were previous smokers while insulin-dependent diabetes is still a significant risk factor (HR 1.65, CI 1.34 - 2.02, p<0.0001) diabetes that is controlled with diet or oral medications is no longer associated with increased risk of major amputation (HR 0.96, CI 0.74 - 1.24, p=0.7611) and in current smokers there is no significant increase in risk of major amputation with either level of diabetes: hazard ratios of 0.86 (95% CI 0.70 - 1.05, p=0.1275) and 1.13 (95% CI 0.94 - 1.36, p=0.1816) for diet/oral medication controlled diabetics and insulin dependent diabetics respectively. A naive interpretation might be that this indicates that smoking has a protective or mitigating effect on diabetes but when we consider that smoking is also a significant risk factor for major amputation (figure 7), we suggest that this effect may represent a saturation of risk or a ceiling effect so that in the presence of one strong risk factor, a second strong risk factor (perhaps one that works along overlapping pathways) doesn't have room to further increase the risk.

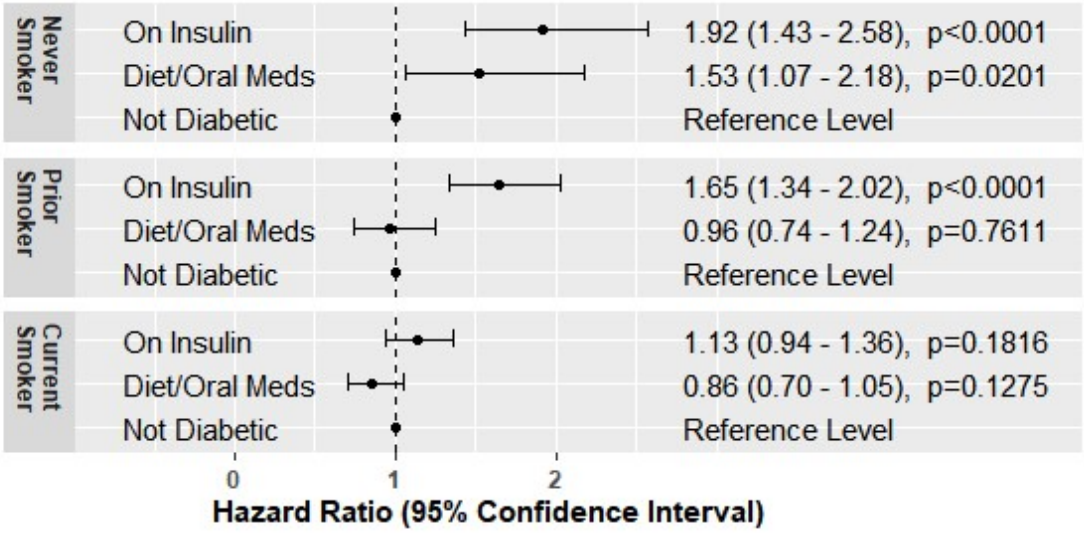

Figure 6: Hazard ratio of major amputation and 95% confidence interval for diabetics on insulin or on diet/oral medications only compared to non-diabetics undergoing elective procedures and adjusted for smoking interaction.

Preoperative smoking status was found to have significant interactions with time from index surgery, diabetes and age. We will calculate hazard ratios for major amputation by smoking status at 1 year after surgery for 65 year old patients (mean age rounded to nearest 5) and stratified by diabetes. As shown in figure 7, current and prior smoking status significantly increases risk of major amputation for non-diabetics with hazard ratios of 2.10 (95% CI 1.50 - 2.93) and 1.42 (95% CI 1.02 - 1.96) respectively, while there is not a significant increase in risk for either level of smoking in the diabetics on diet/oral medications and diabetics on insulin.

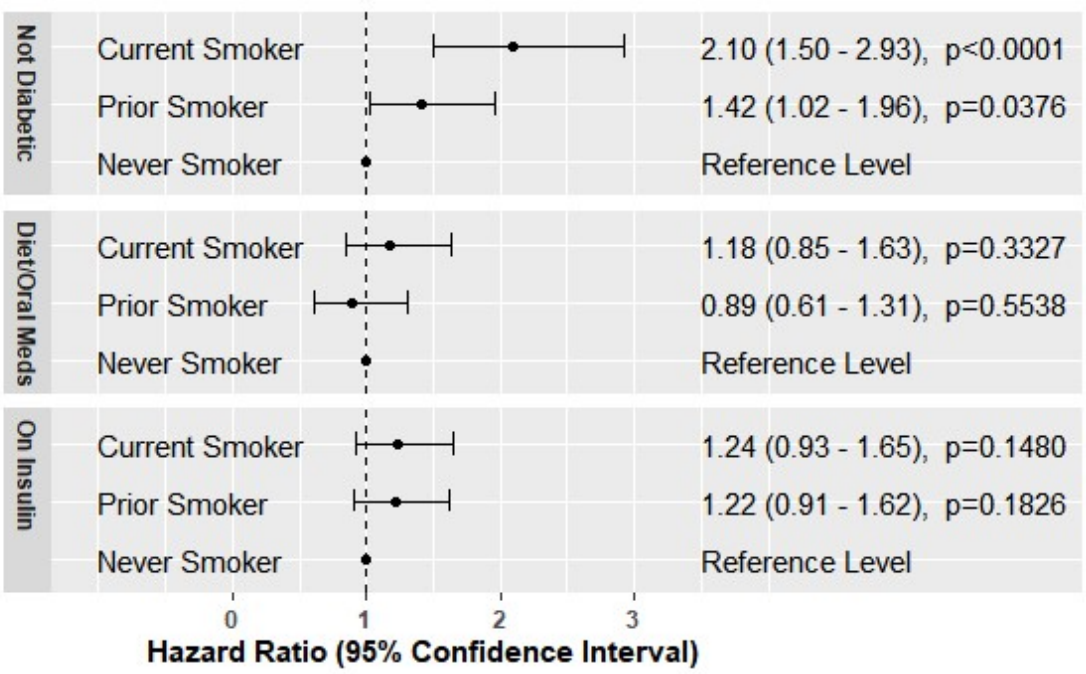

Figure 7: Hazard ratio for major amputation and 95% confidence interval for current and prior smokers compared to never smokers at age 65 and one year after index procedure and adjusted for interaction with diabetes.

Race was found to have a significant interaction with ambulatory status before index procedure. Hazard ratios of major amputation by race are calculated for ambulatory and non-ambulatory patients with white race as the reference level. As shown in figure 8,

black or other non-white race significantly increases the risk of major amputation in ambulatory patients with respective hazard ratios of 1.60 (1.39 - 1.85) and 1.62 (1.36 - 1.92) while in non-ambulatory patients there is no significant difference in risk by race.

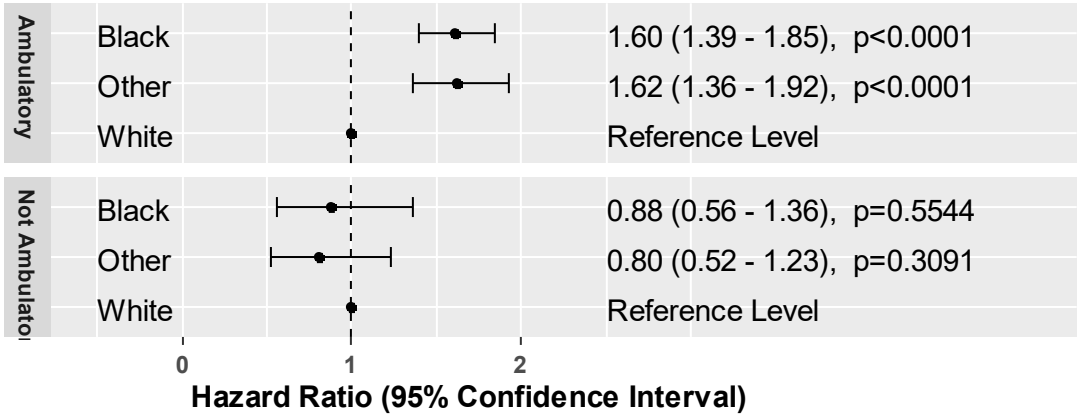

Figure 8: Hazard ratio for major amputation and 95% confidence interval for black and other race compared to white (non-Hispanic) race and adjusted for ambulatory status.

Degree of ischemia was found to have a significant interaction with time from procedure and whether or not the index procedure was elective. Hazard ratios for major amputation by ischemic status on presentation are calculated for elective versus urgent/emergent surgery at 1 year from procedure. As shown in figure 9, presence of tissue loss or rest pain significantly increases the risk of major amputation in patients undergoing elective surgery compared to those without symptoms of CLTI with respective hazard ratios of 2.30 (95% CI 1.79 - 2.96) and 1.76 (95% CI 1.35) but this effect is lost in patients undergoing urgent or emergent surgery.

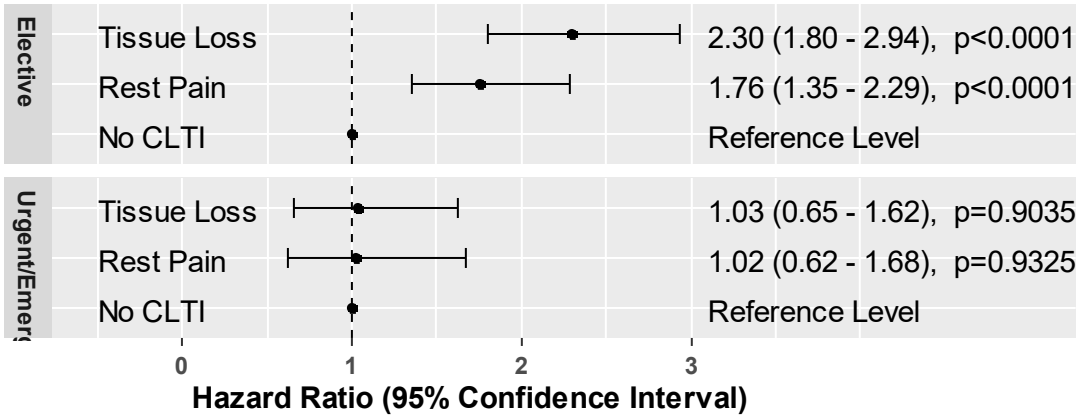

Figure 9: Hazard ratio of major amputation and 95% confidence intervals for presentation with tissue loss or rest pain compared to presentation without chronic limb threatening ischemia (CLTI) and adjusted for whether the index surgery was elective.

Across diabetes, smoking status, race and ischemia we note a common pattern of a decrease in the potency of the risk factor in the presence of another specific risk factor.

DISCUSSION

In the absence of an appropriately designed randomized clinical trial it is difficult to directly compare different therapeutic approaches, especially when a novel alternative is to be compared to a well-established treatment. It can be difficult to even design such a trial because of the ethical implications of randomizing patients to a potentially inferior therapy. Many options exist to leverage large cross-sectional datasets and statistically minimize the bias introduced by the lack of randomization in order to estimate the effects of different treatments but this approach requires that information on the therapeutic options to be compared exist in such a dataset. Large prospective databases might have adequate numbers but lack information on novel treatment options while smaller case series and clinical trials which include newer therapeutic approaches lack the numbers for higher level model fitting. This project represents a strategy to combine the strength in numbers in VQI database with the data on the alternative therapy administered in the MOBILE trial to compare distal bypass surgery with the MarrowStim autologous cell product using a model to account for baseline differences in risk factors between the groups. While powerful in its potential to provide at least a preliminary indication of relative efficacy, there are also a number of limitations inherent to both the data and this analytic approach.

The VQI Infra-Inguinal Bypass registry included information on tens of thousands of bypass procedures but as with any prospectively collected database there are a significant number of missing data points, likely to due to a combination of failure to capture, failure to record and loss to follow up. Specifically, amputation status was missing on 27% of patients. While the Social Security Death Index (SSDI) provides a

mechanism for capturing the majority of mortality, amputation status was only assessed during follow up interactions and thus was more sensitive to both attrition and early mortality since a patient whose death is noted at 6 months in the Social Security Death Index is significantly less likely to receive their 12 month follow up phone call. For this reason, a combined end-point of death or amputation was not considered as any inference that included mortality but censored the patients with missing amputation status would be significantly biased.

Another aspect of the VQI data is the clustering created by the different participating centers. Statistical approaches which account for clustering were used where available but the Gray's test used to compare groups within strata in the non-parametric analysis of outcomes after distal bypass with prosthetic conduit does not account for clustering in the data. An alternative approach was recently proposed which does not assume within cluster independence using a nonparametric cluster bootstrap and might be included in any future work with clustered data instead of Gray's test.[25]

In the second portion of the analysis, a potentially significant source of bias comes with pooling data from different sources. While the variables considered in model selection were collected for both groups, variation in the methods of collection is probable and unmeasured cofounders almost certainly exist. For example, patients who participate in clinical trials might be expected to have more follow-up visits and closer scrutiny compared to those who receive routine post-operative care.

Additionally, there are the problems inherent in the small sample size of the MOBILE trial. With overall just 155 limbs enrolled, covariate estimates will be almost entirely driven by the 17,000 patients in the VQI (except for the levels of treatment

unique to the MOBILE group). This is expected since the power contributed by the larger dataset was the purpose behind the selection of this analytic approach but it does assume that the effect of a given covariate estimated from the VQI data is not significantly different from that which would be obtained if we had a similar number of MOBILE patients (e.g. that the effect of prior bypass on risk of major amputation is similar in the VQI and MOBILE patients).

And finally, because of the 3:1 randomization scheme in the MOBILE trial there are just 33 patients in the best medical management group. The consequent and expected imprecise estimates of effect in this group as reflected by the wide confidence intervals is the reason that this otherwise obvious choice for a control group was not used.

In the non-parametric analysis of the VQI data, the estimated cumulative incidence of major amputation following distal bypass was found to be highest in patients who had a non-autologous biologic conduit so this cohort was selected as a reference group. Risk of major amputation is lowest in those who had a GSV bypass and prosthetic conduit fell between the two. These difference cannot entirely be attributed to the conduit type however because of the unequal distribution of risk factors across these groups as outlined in table 1 but these findings are nevertheless a reflection of actual outcomes of distal bypass by conduit as they are performed across the centers participating in VQI in the last decade. This supports the use of GSV first whenever possible and strongly suggests that second choice for distal bypass should be prosthetic, although individual factors such as infection risk must still be considered; the high estimated incidence of amputation in the biologic group at one year may prompt careful consideration of

concomitant risk factors and frank discussions with patients regarding their expectations for surgery and the option of early amputation.

The stratification by some common risk factors for the prosthetic cohort was provided to inform similar consideration for those patients who are candidates for distal bypass without adequate GSV. Specifically, such bypasses with a tibial vessel for outflow or in patients who are not ambulatory have an estimated risk of major amputation greater than 20% at one year. These estimations of risk should not be applied indiscriminately to individual patients as they include the contribution of the spectrum of risk factors found in vascular patients and actual risk may be higher or lower for any individual. But they do reflect the cumulative incidence across almost four thousand procedures and reflect the average risk in that population. The level of risk which is acceptable in any specific case remains a shared decision between surgeon and patient.

Further, while we considered the three main conduit types, not all distal reconstruction options were considered in this analysis including the role of heparin bonding or vein cuffs (which were included in the prosthetic cohort) or composite or non-GSV autologous vein options (which were excluded) and remain to be addressed in a future study.

The treatment option which was compared to those three main conduit types in this project was autologous cell therapy with MarrowStim, which is composed of never frozen and minimally processed nucleated bone marrow cells from the individual patient and which was administered to 119 limbs as a part of the MOBILE trial. A cox proportional hazards model was fit as described above to identify and account for the role of significant risk factors on the incidence of major amputation and estimate the effect of

treatment with either distal bypass with GSV or prosthetic or administration of MarrowStim or best medical management compared to bypass with non-autologous biologic conduit. This approach resulted in findings consistent with what is already known about risk factors for amputation including severity of ischemia on presentation, age, race, diabetes, ambulatory status, urgency of revascularization procedure and smoking history. Treatment was also significant and was notably found to interact with age of the patient. The hazard ratios estimated for distal bypass with GSV or prosthetic compared to biologic were relatively constant with increasing age so this association appears to be largely driven by the MarrowStim therapy which was estimated to significantly decrease the risk of major amputation in younger patients (age groups 55 and 60) compared to both biologic and prosthetic bypass, was still significant compared to biologic at 65 years old but lost efficacy compared to either in older patients (age group 70). This decrease in benefit with increasing age may be attributed to either recipient or donor specific factors. Although these are one and the same in this case, recipient factors are related to the body's ability to respond to cell therapy which is thought to be mediated by recruitment and enhancement of pathways related to angiogenesis, mitigation of oxidative stress and mitochondrial function, pathways that may be less responsive and functional in older patients. Donor factors include the issues related to extracting the cell product from older bone marrow which might be expected to contain more senescent cell lines and decreased expression of proliferative factors.

Overall, these findings will inform the design of the MOBILE trial's successor which may include less rigorous exclusion criteria to include not just no option patients but those with suboptimal options such as distal bypass with cadaveric vein or artery. The

use of an allogeneic cell product from a young health donor may also be considered rather than autologous cells, decreasing the procedural burden on the patient.

Finally, while all the primary aims of the project have been addressed, there remains the finding related to the significant interactions between risk factors, notably the mitigation of a strong risk factor in the presence of a second risk factor. The significance of insulin-dependent diabetes vanished in the presence of current smoking and vice versa. The risk associated with non-white race is noted in ambulatory patients but not in the non-ambulatory and the increasing risk of amputation with worsening ischemia on presentation is only noted for elective surgeries, not for those classified as urgent/emergent (although for the latter the definition may be called into question as there were a surprising number of patients without CLTI that fell in that category).

Regardless, this finding is interesting and we suggest that implication of a mitigating effect would be misleading. Rather, this may represent evidence for a saturation of risk or for a common pathway. For example, diabetes was found to interact with smoking status but did not significantly interact with race. This may be because current smoking and insulin-dependent diabetes are both stronger risk factors than black or other non-white race, so in combination encounter a theoretical "ceiling" of risk sooner than the combination of insulin dependent diabetes with black race. This interpretation would suggest that all risk factors interact to some extent, including race with diabetes. There may be some truth to this as even though that interaction did not reach significance at a level of 0.05 with a p-value 0.06154, it was close.

An alternate though not mutually exclusive interpretation would be a common pathway; that is that the downstream physiologic mechanism (e.g. endothelial

dysfunction) by which insulin dependent diabetes increases the risk of amputation overlaps with that of current smoking so that if the pathway is already fully engaged there can be no additional activation by the other risk factor. This is supported by the distinct clusters of interactions among the risk factors, suggestive of multiple unique pathways of effect.

Overall, the project has opened several avenues of further investigation. A more exhaustive analysis of outcomes by conduit might include composite and non-GSV autologous vein conduits as well as a common modification of prosthetic bypass the addition of a vein cuff. Specifically, the addition of above the knee bypasses to the cohort and an investigation of the contribution of target vessel is indicated as the classic cutoff of below versus above the knee may be both too restrictive and too simplistic. This less exclusive dataset could also be used to determine if the previously noted interactions remain significant with additional observations and judicious non-parametric stratification could be used to determine if there is also indication of such interactions without relying on models.

APPENDIX

Supplementary figures

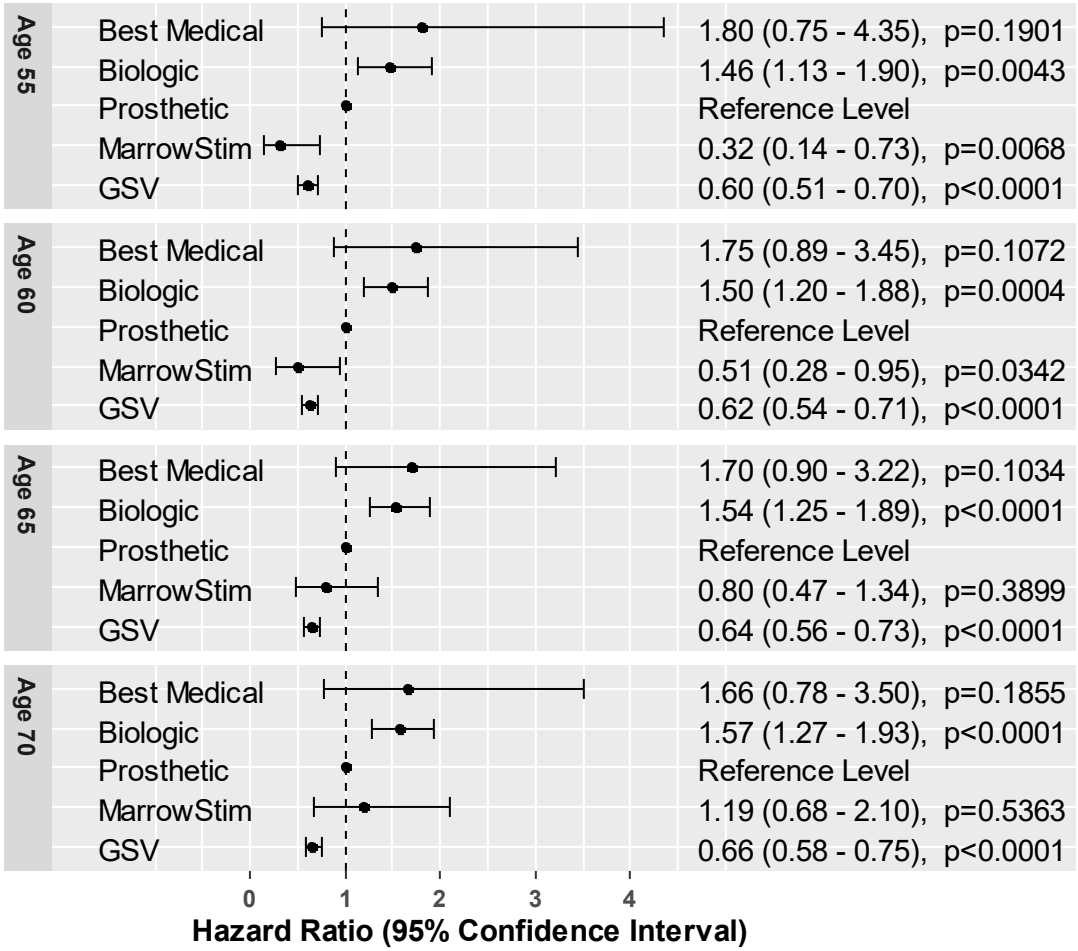

Figure S-1: Hazard ratio of major amputation and 95% confidence interval for each treatment group compared to prosthetic bypass and adjusted for age.

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CURRICULUM VITAE

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Publications/Posters:

- Abstract 06/2020
  Poster at Society for Vascular Surgery 2020 Annual Meeting (online)
  Allogeneic mesenchymal stromal cells promote muscle fiber regeneration in a diabetic mouse model of critical limb ischemia
  Justin R. King, Katherin Leckie, Amy Y. Sato, Teresita M. Bellido, Marlee Yancey, Leni Moldovan, Michael P. Murphy, Steven J. Miller

- Abstract 05/2020
  Top abstract at the 4th Annual Riley Hospital Surgical Research Day

Pre-Hospital High Volume Crystalloid Resuscitation Increases Mortality in Pediatric Traumatic Brain Injury Patients
Brian Hosfield MD, Katherin Leckie MD, Cody Jones BA, Alyson Baker MD, Jodi Raymond MPH, Courtney Rowan MD, Laurie Ackerman MD, Matthew Landman MD, MPH, FACS, FAAP

- Book section 05/2020
  Chronic Venous Disease
  In Venous Disease in Mulholland and Greenfield's Surgery: Scientific Principles & Practice, 7th edition, edited by Justin B. Dimick, Gilbert R. Upchurch, Jr, Hasan B. Alam, Timothy M. Pawlik, Mary Hawn, and Julie Ann Sosa
  Katherin E Leckie, Michael C. Dalsing

- Journal article 02/2020
  Deletion of Socs3 expression in aortic smooth muscle cells ameliorates aortic dissection
  JACC: Basic to Translational Research
  Michael Murphy, Justin King, Katherin Leckie

- Abstract 03/2019
  Presentation at Midwestern 2019
  Allogeneic Mesenchymal Stem Cells Induce Regulatory T-Cells and Suppress Aneurysm Inflammation: Interim Results of the Phase I ARREST Trial
  Katherin Leckie, Linden L. Green, Justin R. King, Keisin S. Wang, MD, Raghu L. Motaganahalli, Andres Fajardo, Michael P. Murphy, John G. Maijub

- Abstract 03/2019
  Presentation at Midwestern 2019 (presented by Justin King)
  The MicroRna Cluster of Mir-15a,-27a,and-92a Are Associated with Diminished Il-10 Levels and Decreased Frequency and Immune Suppressor Function of Type-1 Regulatory T Cells in Patients with Abdominal Aortic Aneurysms

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  Katherin E. Leckie, Linden A. Green, Keisin S. Wang, Ashley R. Gutwein, Raghu L. Motaganahalli, Andres Fajardo, John G. Maijub, Michael P. Murphy

- Abstract 06/2018
  Poster at SVS VAM 2018 (presented by Justin King)
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  Justin R. King, Katherin Leckie, David Rollins, Lavaraj Timsina, Aaron Franke, Christa Dixon, Lynda Nelson, Raghu L. Motaganahalli, MD

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