Sex-based differences in the inflammatory profile of peripheral artery disease and the association with primary patency of lower extremity vein bypass grafts

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Objective: **This study was conducted to determine if there are sex-based differences in the inflammatory phenotype of patients undergoing lower extremity bypass (LEB) and if they correlate with clinical outcomes.**

Methods: **This was a retrospective analysis of a prospective cohort of 225 patients (161 men and 64 women) who underwent autogenous vein LEB between February 2004 and May 2008. Fasting baseline blood samples were obtained before LEB, and the inflammatory biomarkers high-sensitivity C-reactive protein (CRP) and fibrinogen were assessed. All patients underwent ultrasound graft surveillance. CRP levels were dichotomized at 5 mg/L and fibrinogen levels at 600 mg/dL.**

Results: **There were no significant differences in age, race, history of hypertension or diabetes mellitus, body mass index, or coronary artery disease between men and women. Men were more likely to be current smokers ($P = .02$), have a history of hypercholesterolemia ($P = .02$), and be taking statins ($P = .02$). Women were more likely to present with critical limb ischemia ($P = .03$) and had higher median baseline CRP levels (5.15 mg/L; interquartile range [IQR], 1.51-18.62 mg/L) than men (2.70; IQR, 1.24-6.98 mg/L; $P = .02$). Median follow-up was 893 days (IQR, 539-1315 days). A multivariable Cox proportional hazards model for primary vein graft patency showed a significant interaction between sex and CRP ($P = .03$) and fibrinogen ($P = .02$). After adjustment for key covariates, primary vein graft patency was significantly less in women with CRP >5 mg/L compared with women with CRP <5 mg/L ($P = .02$). No such difference was seen in men ($P = .95$). Primary graft patency was also decreased in women with fibrinogen >600 mg/dL vs women with fibrinogen <600 mg/dL ($P = .002$); again, this pattern was not evident in men ($P = .19$).**

Conclusions: **Women undergoing LEB for advanced peripheral artery disease have a different inflammatory phenotype than men. Elevated baseline levels of CRP and fibrinogen are associated with inferior vein graft patency in women but not in men. These findings indicate an important interaction between sex and inflammation in the healing response of vein grafts for LEB. Women with elevated preoperative CRP and fibrinogen levels may benefit from more intensive postoperative graft surveillance protocols. (J Vasc Surg 2012;56:387-95.)**

Although women have lower overall rates of cardiovascular disease (CVD) than men until the seventh decade of life,[1] several population studies have demonstrated higher age-adjusted rates of peripheral artery disease (PAD) in women.[2-4] Also, women who undergo lower extremity bypass (LEB) procedures appear to have increased rates of wound complications and lower rates of graft patency than men.[5-7] The potential reasons for these poorer outcomes in women include smaller conduit and target vessels, more advanced disease, older age at presentation, and a difference in the underlying state of inflammation in women than in men.

Clinical studies have established that chronic inflammation is a strong risk factor for future atherothrombotic disease. C-reactive protein (CRP) is an acute-phase protein that is elevated in individuals at high risk for myocardial infarction,[8-10] stroke,[9,11] and cardiovascular death.[9] CRP levels are also elevated in individuals with PAD,[12,13] and higher CRP levels have been associated with the risk and progression of PAD.[14,15] Recent studies have also suggested that preoperative CRP levels are predictive of adverse outcomes after vascular procedures, including LEB surgery.[16-18]

Fibrinogen, like CRP, is an acute-phase reactant.[19] In addition, fibrinogen has important hemostatic properties, as it affects platelet and red cell aggregation as well as endothelial function.[19] As a result, high levels of fibrinogen

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in plasma might reduce blood flow, predispose to thrombosis, and enhance atherogenesis. Fibrinogen is also critical in inflammation; in an ex-vivo study, fibrinogen was important in mediating leukocyte adhesion to human vein grafts.[20] Fibrinogen predicts the severity of PAD[17,21] and serves as a marker for the future development of PAD.[22,23] Moreover, some studies have demonstrated a positive association between plasma fibrinogen levels and subsequent graft stenosis and occlusion.[24,25]

Several population studies have demonstrated higher levels of CRP[26,27] and fibrinogen[28-30] in women compared with men, but the clinical significance of this is not known. Although many studies have evaluated the association between inflammatory markers and CVD outcomes, fewer have investigated the role of inflammation and graft patency after LEB procedures. Moreover, it is unclear whether the differences in the underlying inflammatory profiles between men and women are important in the outcome after LEB. To address this gap in research, our current study investigated whether sex-based differences exist in the baseline inflammatory markers of patients undergoing LEB and if these biomarker levels are associated with primary vein graft patency.

METHODS

Study design. This was a prospective cohort study sponsored by the National Institutes of Health and conducted at three Boston academic medical centers. The study sought to determine the relationship between inflammation and outcomes after LEB using autogenous vein grafts. Enrollment began in February 2004 and ended in May 2008 and included 225 patients. Details of patient selection, inclusion and exclusion criteria, and operative procedure have been previously described.[17,31] Patients were excluded if they were treated with a prosthetic or nonautologous vein graft, had a history of a hypercoagulable state, or had a concurrent significant event ≤30 days before the index bypass operation (ie, myocardial infarction, stroke, or major surgical procedure). The study excluded patients with deep space infections of the foot or active infection. Although patients with small ulcers or those with dry gangrene were included in the study, those with ulceration or gangrene requiring operative debridement were excluded.

Blood collection and biomarker measurement. Blood was collected from the fasted patient on the morning of the LEB procedure. All samples were immediately centrifuged at 3000 rpm and stored at −80°C until analysis. All samples were analyzed in batches at a core laboratory to minimize variability. CRP levels were determined by a high-sensitivity immunoturbidimetric assay on a Hitachi 917 analyzer (Roche-Diagnostics, Indianapolis, Ind) using reagents and calibrators from Denka Seiken (Niigata, Japan), with a sensitivity of 0.03 mg/L. Day-to-day variations of the assay at differing concentrations were <3%. Fibrinogen was measured using an immunonephelometric technique on the Behring BNII analyzer (Dade Behring, Newark, Del). Other inflammatory and metabolic biomarkers were measured in the parent study and have been previously reported.[31]

Covariates. Race and sex were assessed by self-report. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or if the patient was taking prescription medications for hypertension. Hypercholesterolemia and diabetes mellitus were present if the patient was taking prescribed medications or if the diagnosis was self-reported. Active smoking was defined by a positive answer to the question "Do you now smoke cigarettes?" A former smoker was defined as one who had smoked >100 cigarettes in his or her life but had not smoked in 30 days. Chronic kidney disease was defined by an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease Study equation.[32] Body mass index (BMI) was calculated as kg/m^2.

CRP levels were dichotomized at 5 mg/L and fibrinogen levels at 600 mg/dL. Although the American Heart Association and Centers for Disease Control and Prevention guidelines support measurement of CRP for risk stratification and consider CRP >3 mg/L to be a high cardiovascular risk, the median value in our overall cohort was nearly 3 mg/L, and the median value in women was >5 mg/L. We dichotomized at CRP >5 mg/L because this value has previously been shown to represent a high-risk subgroup in a stroke cohort,[33] and patients with CRP >5 mg/L have impaired early vein graft luminal remodeling.[34] CRP >5 mg/L also represented the upper limit of our core laboratory's reference range. The standard reference values for fibrinogen range from 150 to 450 mg/dL.[19] The median value in our cohort was 480 mg/dL (interquartile range [IQR], 401-585 mg/dL). We chose to dichotomize at 600 mg/dL because this represented the top quartile of values.

Operative procedure and surveillance. All patients underwent LEB using autogenous vein, with ipsilateral great saphenous vein (GSV) as the conduit of first choice. In the absence of suitable ipsilateral vein, the contralateral GSV or arm vein was used. Routine postoperative follow-up included a complete vascular examination by the attending surgeon and a standard duplex ultrasound examination of the bypass graft in an accredited vascular laboratory at 1, 3, 6, 9, and 12 months. After 12 months, follow-up visits occurred every 6 months. All clinical and graft-related events were recorded prospectively. Although the decision to intervene on a failing graft was not standardized across all surgeons, as a general rule, all patients underwent graft intervention for all hemodynamically significant stenoses detected on surveillance ultrasound imaging. The type of graft reintervention was left to the discretion of the operating surgeon.

Definition of primary patency. Loss of primary patency included any type of graft revision (balloon angioplasty, patch angioplasty, or interposition graft) when a graft stenosis was detected by duplex ultrasound imaging or

Table I. Demographic characteristics of the cohort

Variable[a]	*Men (n = 161)*	*Women (n = 64)*	P
Age, years	67.0 ± 10.4	69.3 ± 12.0	.14
Black race	9 (5.6)	8 (12.5)	.09
Current smoker	67 (41.6)	16 (25.0)	.02
Diabetes	88 (54.7)	29 (45.3)	.21
Body mass index, kg/m^2	27.9 ± 5.4	29.6 ± 8.6	.08
Hypertension	133 (82.6)	57 (89.1)	.23
Coronary artery disease	90 (55.9)	28 (43.8)	.10
eGFR, mL/min/1.73 m^2	78.2 ± 32.9	70.8 ± 39.3	.15
Hypercholesterolemia	123 (76.4)	39 (60.9)	.02
Statin therapy	135 (83.9)	45 (70.3)	.02
Critical ischemia	88 (54.7)	45 (70.3)	.03
Composite vein	21 (13.0)	14 (21.9)	.10
CRP, mg/L	2.70 (1.24-6.98)	5.15 (1.51-18.62)	.02
CRP >5 mg/L	56 (34.8)	34 (53.1)	.01
Fibrinogen, mg/dL	498 ± 159	536 ± 211	.16
Fibrinogen >600 mg/dL	38 (23.6)	21 (32.8)	.16

CRP, C-reactive protein; *eGFR,* estimated glomerular filtration rate.
[a]Continuous data are shown as the mean ± standard deviation and median (IQR) and categoric data as number (%).

other imaging modality. Loss of primary patency was also noted if there was documentation of occlusion of the vein graft without revision.

Statistical analysis. All statistical analyses were performed using Stata/SE 10.1 software (StataCorp, College Station, Tex). The clinical characteristics and biomarker values of the cohort were compared for men and women and are presented as proportions for categoric characteristics and as mean ± standard deviation (SD) or median and IQR for continuous characteristics. Continuous variables between groups were compared with the Student *t*-test; if the distribution was not approximately normal, the Wilcoxon rank-sum test was used. Proportions between groups were compared with the Pearson χ^2 test. A value of $P \leq .05$ was considered statistically significant.

Variable selection for multivariable models. The bivariate associations between clinical variables, graft characteristics, and biomarker levels with primary graft patency were assessed for the overall cohort using a Cox proportional hazards model. Variable selection was based on this primary analysis. Any variable with a value of $P < .30$ was included in the subsequent multivariable models. Interactions between sex and biomarker levels, race and biomarker levels, sex and race, and sex and critical ischemia were also assessed and further analyzed if the *P* value for interaction was <.30. A multivariable Cox proportional hazards model was used to evaluate primary patency in men and women. We did not include CRP and fibrinogen in the same multivariable models because it is scientifically plausible that CRP and fibrinogen are potential mediators for one another in the pathway to graft failure; hence, including both biomarkers in the same model would result in overadjustment. Indeed, CRP and fibrinogen were highly correlated in our cohort (Pearson correlation coefficient, 0.56; $P < .001$).

RESULTS

The 225 patients (28% women) were a mean age of 68 ± 11 years and 8% were black. There were no significant differences in age, race, diabetes mellitus, BMI, eGFR, history of hypertension, and coronary artery disease between men and women (Table I). Men were more likely to be current smokers ($P = .02$), have a history of hypercholesterolemia ($P = .02$), and be on statin therapy ($P = .02$) compared with women (Table I). Women were more likely to present with critical limb ischemia ($P = .03$) and had significantly higher baseline CRP levels ($P = .02$) than men (Table I).

The indication for LEB surgery in 133 patients (59%) was critical limb ischemia, and 73 patients (32%) had evidence of distal tissue loss. Bypass graft placement in 190 patients (84%) was with a single-segment GSV, whereas the remainder underwent construction of bypass graft using composite GSV or arm vein. A previous ipsilateral bypass graft had failed in 22 patients (10%). There were no differences in the type of conduit used, primary vs redo bypass, or type of outflow vessel (popliteal, tibial, or pedal artery) in women compared with men.

The median CRP level in the entire cohort was 2.98 mg/L (IQR, 1.28-12.25). The median CRP level was higher in women (5.15; IQR, 1.51-18.62 mg/L) compared with men (2.70; IQR, 1.24-6.98 mg/L; $P = .02$; Table I). The mean fibrinogen level in the cohort was 509 ± 176 mg/dL and was not significantly different in women (536 ±211 mg/dL) compared with men (498 ± 159 mg/dL; $P = .16$; Table I). In a multivariable analysis that included sex, race, age, BMI, coronary artery disease, diabetes, statin use, critical ischemia, current smoking, and eGFR, sex ($P = .04$) and critical ischemia ($P < .001$) were both independently associated with baseline CRP level >5 mg/L. In a similar model for fibrinogen, diabetes ($P =$

Table II. Bivariate associations between predictors and primary patency, stratified by sex

Variable	*Men* *HR (95% CI)*	P	*Women* *HR (95% CI)*	P
Age	1.00 (0.98-1.03)	.72	1.00 (0.97-1.04)	.90
Black race	2.39 (0.95-6.06)	.07	2.80 (1.11-7.06)	.03
Smoking	1.06 (0.60-1.85)	.85	0.94 (0.39-2.25)	.89
Diabetes	1.80 (1.01-3.21)	.05	1.17 (0.53-2.57)	.70
Body mass index	0.97 (0.91-1.02)	.22	1.01 (0.97-1.06)	.57
Hypertension	1.01 (0.49-2.08)	.97	3.18 (0.43-23.54)	.26
Coronary artery disease	1.08 (0.62-1.88)	.79	1.64 (0.75-3.61)	.22
Hypercholesterolemia	0.84 (0.44-1.57)	.58	2.36 (0.94-5.91)	.07
Statin therapy	0.68 (0.35-1.32)	.25	1.07 (0.45-2.56)	.88
eGFR	1.00 (0.99-1.01)	.66	1.00 (0.99-1.01)	.74
Critical ischemia	2.02 (1.14-3.63)	.02	1.42 (0.59-3.40)	.43
Composite vein	1.41 (0.67-3.03)	.36	1.93 (0.85-4.36)	.12
CRP > 5 mg/L	1.35 (0.76-2.39)	.30	2.64 (1.16-6.02)	.02
Fibrinogen >600 mg/dL	0.94 (0.48-1.84)	.87	2.87 (1.29-6.38)	.01

CI, Confidence interval; *CRP,* C-reactive protein; *eGFR,* estimated glomerular filtration rate; *HR*, hazard ratio.

.008) and critical ischemia ($P = .03$) were associated with baseline fibrinogen levels >600 mg/dL.

The median follow-up was 893 days (IQR, 539-1315 days). There were 78 (35%) primary graft failures; this occurred in 27 of 64 women (42%) and in 51 of 161 men (32%; $P = .14$). Of the 78 primary patency events, 26 (33%) were graft thromboses and 52 (67%) were revisions for graft stenosis. Men and women had similar profiles for loss of primary patency, with 10 of 27 thrombotic events (37%) in women and 16 of 51 thrombotic events (31%) in men ($P = .61$). There was no significant difference in the type of primary patency event (stenosis vs thrombosis) by CRP levels ($P = .20$) or fibrinogen values ($P = .48$) in the overall cohort or when analyzed separately by sex. There were 35 secondary graft failures in this cohort, 11 of 64 in women (17%) and 24 of 161 in men (15%; $P = .67$).

The bivariate associations between all of the variables listed in Table I and primary graft patency were evaluated in the entire cohort and then separately for men and women (Table II). In the overall cohort, black race ($P = .002$), diabetes mellitus ($P = .07$), critical ischemia ($P = .009$), composite vein graft ($P = .06$), CRP > 5 mg/L ($P = .02$), and fibrinogen >600 mg/dL ($P = .17$) met the criteria ($P < .30$) for inclusion into the multivariable models. When these bivariate associations between predictors and primary graft failure were stratified by sex, the presence of diabetes mellitus ($P = .05$) and critical ischemia ($P = .02$) were significantly associated with primary graft failure in men. In women, black race ($P = .03$), CRP > 5 mg/L ($P = .02$), and fibrinogen > 600 mg/dL ($P = .01$) were associated with primary graft failure (Table II).

In a multivariable Cox proportional hazards model for primary graft patency for the entire cohort, there was no significant difference in primary vein graft patency in those individuals with CRP >5 mg/L compared with those with CRP <5 mg/L ($P = .11$; Fig 1, *A*). However, there was a statistically significant interaction between sex and CRP levels ($P = .03$). Women with CRP >5 mg/L were significantly more likely to lose primary vein graft patency compared with women with CRP <5 mg/L (hazard ratio [HR], 2.63; 95% confidence interval [CI], 1.15-6.02; $P = .02$), but no such difference was seen in men (HR, 0.98; 95% CI, 0.52-1.85; $P = .95$; Fig 1, *B* and *C*). The findings were similar with respect to secondary graft patency. Women with CRP >5 mg/L were significantly more likely to lose secondary graft patency than women with CRP <5 mg/L ($P = .05$), but no such difference was seen in men ($P = .78$).

There was also a significant interaction between sex and fibrinogen ($P = .02$). In a multivariable Cox proportional hazards model for primary graft patency, there was no significant difference in primary vein graft patency in those with fibrinogen >600 mg/dL compared with those with fibrinogen <600 mg/dL in the overall cohort ($P = .48$; Fig 2, *A*). However, women with fibrinogen >600 mg/dL were significantly more likely to lose primary vein graft patency than women with fibrinogen <600 mg/dL (HR, 4.09; 95% CI, 1.66-10.05; $P = .002$; Fig 2, *B*), but again, no such difference was demonstrated in men (HR 0.62; 95% CI, 0.30-1.27; $P = .19$; Fig 2, *C*). These findings were similar when secondary graft patency was evaluated. Women with fibrinogen >600 mg/dL were significantly more likely to lose secondary vein graft patency than women with fibrinogen <600 mg/dL ($P = .03$), but no difference was demonstrated in men ($P = .45$).

The HRs for loss of primary graft patency, stratified by sex and in separate multivariable models of CRP and fibrinogen, are reported in Tables III and IV, respectively. In the CRP multivariable model for primary graft patency, black race was significantly associated with primary graft loss in men (HR, 2.68; 95% CI, 1.04-6.86; $P = .04$), whereas black race (HR, 3.04; 95% CI, 1.14-8.10; $P = .03$) and CRP > 5 mg/L (HR, 2.63; 95% CI, 1.15-6.02; $P = .02$) were significantly associated with primary graft loss in women (Table III). In the multivariable model with fibrinogen, black race (HR, 2.80; 95% CI, 1.09-7.22; $P = .03$) and critical ischemia (HR, 1.87; 95% CI, 1.00-3.50; $P = .05$) were significantly associated with primary graft loss in

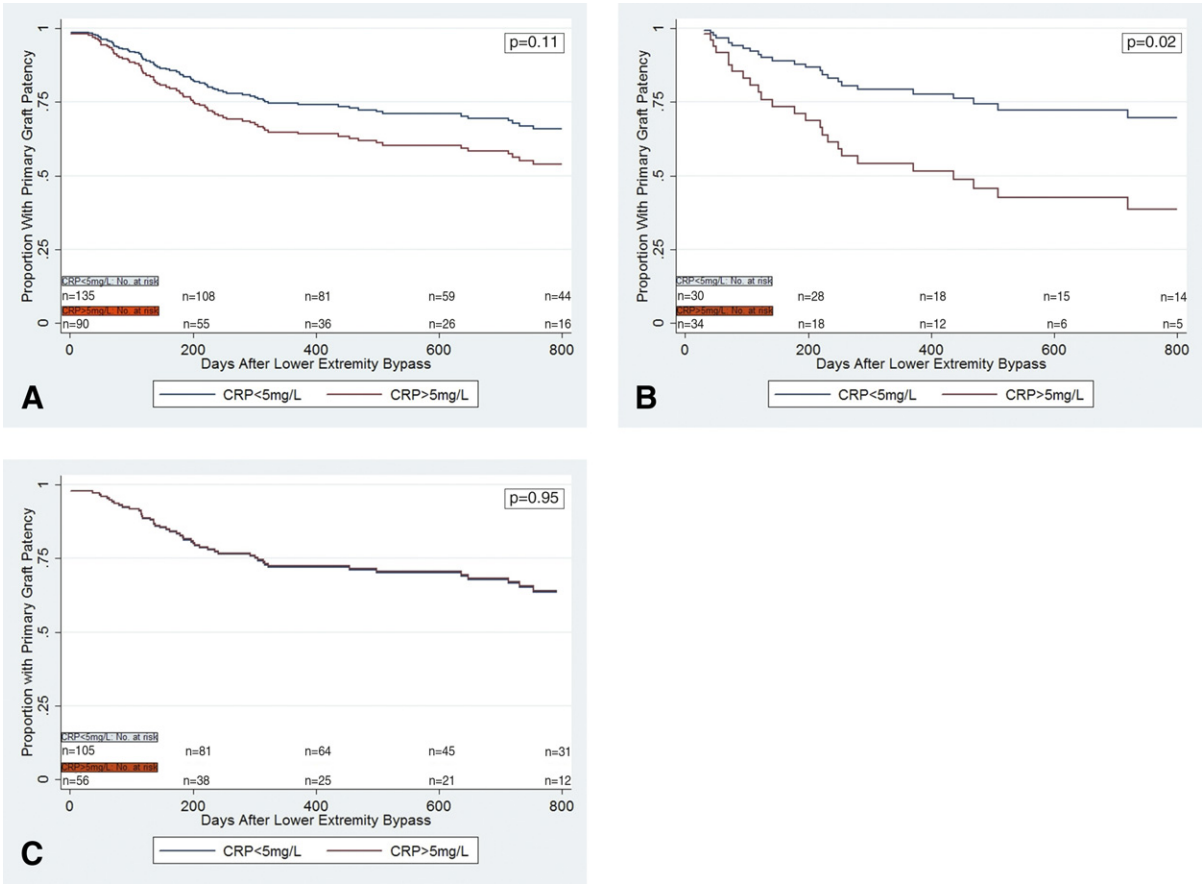


Fig 1. A, Multivariable Cox regression curves show primary graft patency for **(A)** all patients, for **(B)** women, and for **(C)** men undergoing lower extremity bypass surgery with autogenous vein based on C-reactive protein (*CRP*) values.

men, whereas in women, black race (HR, 4.22; 95% CI, 1.53-11.64; $P = .005$) and fibrinogen >600 mg/dL (HR, 4.09; 95% CI, 1.66-10.05; $P = .002$) were significantly associated with primary graft loss (Table IV).

DISCUSSION

This prospective study of patients with advanced PAD undergoing LEB with autogenous vein demonstrated that elevated baseline levels of CRP and fibrinogen were each associated with graft failure in women but not in men. We found a significant interaction between sex and both CRP and fibrinogen in the primary patency rate of LEB grafts, suggesting a different underlying inflammatory profile in men and women who present with severe PAD.

In our study, women had significantly higher baseline levels of CRP compared with men. This is not surprising, as several large cohort studies have demonstrated differences in CRP levels by both sex and race. The Multiethnic Study of Atherosclerosis (MESA) cohort of 6814 men and women aged 45 to 84 years found substantially higher median CRP levels in women than in men, despite accounting for estrogen use, BMI, and other confounding variables.[26] This sex difference was maintained across all ethnic subgroups. In the Dallas Heart Study, women had significantly higher CRP levels than men, and black patients had significantly higher levels than white patients.[27] These findings remained significant after adjustment for BMI and traditional cardiovascular risk factors, as well as after exclusion of individuals taking statins and estrogens. Our study population is limited with regards to racial diversity; therefore, we were unable to examine the potential synergies between sex, race, and inflammation. Although men and women differed in several baseline factors, it is noteworthy that female sex was independently correlated with CRP level even after adjustment for multiple covariates.

Previous studies have demonstrated CRP to independently predict future CVD events in both men and women.[8-10] However, subgroup observations from both the Cardiovascular Health Study (CHS) and the Rural Health Promotion Project demonstrated the risks of vascular disease associated with CRP were greater for women than for men.[35] Similarly, in the Women's Health Study, the adjusted relative risk for either myocardial infarction or stroke in women with the highest quartile of CRP was 5.5 compared with 2.8 in men participating in the Physicians'

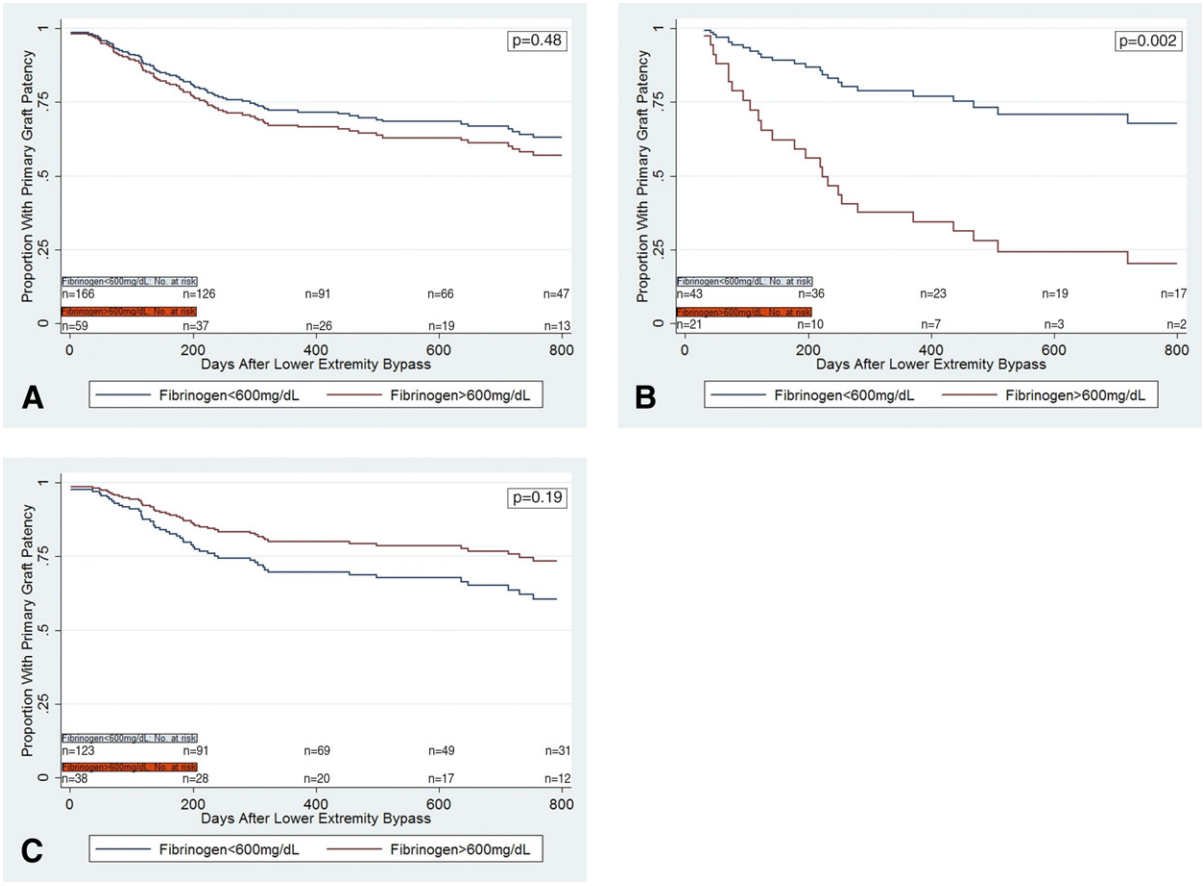


Fig 2. Multivariable Cox regression curves for primary graft patency for **(A)** all patients, **(B)** for women, and **(C)** for men undergoing lower extremity bypass surgery with autogenous vein, based on fibrinogen levels.

Table III. Multivariable Cox proportional hazards model for primary graft patency, including C-reactive protein (*CRP*), stratified by sex

Variable	*Men* *HR (95% CI)*	P	*Women* *HR (95% CI)*	P
Black race	2.68 (1.04-6.86)	.04	3.04 (1.14-8.10)	.03
Diabetes	1.64 (0.88-3.04)	0.12	1.10 (0.47-2.57)	0.82
Composite vein	1.21 (0.56-2.64)	0.63	1.85 (0.78-4.39)	0.16
Critical ischemia	1.72 (0.88-3.34)	0.11	1.16 (0.48-2.83)	0.74
CRP >5 mg/L	0.98 (0.52-1.85)	0.95	2.63 (1.15-6.02)	.02

CI, Confidence interval; *HR*, hazard ratio.

Health Study.[8,9] Although CRP is a strong and independent risk factor for adverse cardiovascular outcomes in men and women, these findings suggest that women with elevated CRP may be at a relatively higher risk for a cardiovascular event compared with men. Few studies have been performed examining the association between CRP and LEB graft patency,[16-18] and none of these examined results by sex.

Recent work suggests there may be an important relationship between systemic inflammation and the early remodeling changes in LEB grafts; specifically, the early (1-month) venous dilation response to arterialization appears to be impaired in patients with elevated baseline CRP levels.[34] This suggests a possible uncoupling between hemodynamic stress and vessel remodeling. If true, such impaired early remodeling may likely have greater clinical impact in the setting of smaller conduit vessels. In this study, we found that the divergence of loss of primary patency, by preoperative biomarker level, primarily occurred within the first year, further supporting this idea. This hypothesis will need to be formally examined in future and ongoing studies.

Table IV. Multivariable Cox proportional hazards model for primary graft patency, including fibrinogen, stratified by sex

Variable	*Men* *HR (95% CI)*	P	*Women* *HR (95% CI)*	P
Black race	2.80 (1.09-7.22)	.03	4.22 (1.53-11.64)	.005
Diabetes	1.85 (0.98-3.50)	.06	0.92 (0.40-2.10)	.84
Composite vein	1.13 (0.51-2.46)	.77	2.11 (0.92-4.83)	.08
Critical ischemia	1.87 (1.00-3.50)	.05	0.84 (0.33-2.14)	.71
Fibrinogen >600 mg/dL	0.62 (0.30-1.27)	.19	4.09 (1.66-10.05)	.002

CI, Confidence interval; *HR*, hazard ratio.

Fibrinogen levels also differ by sex and race and have been reported to be higher in women than in men[28-30] and in blacks than in whites.[28-30] Fibrinogen levels increase with age, smoking, body size, diabetes, fasting serum insulin, low-density lipoprotein cholesterol, and menopause.[28-30] A meta-analysis of fibrinogen as a CVD risk factor identified six prospective epidemiologic studies, all of which demonstrated that fibrinogen was associated with subsequent myocardial infarction or stroke.[36] Although fibrinogen appears to be a strong CVD risk factor in both men and women, it is unclear from any of these previous studies whether there is a differential association in women compared with men.

We found a strong interaction between sex and fibrinogen with regards to vein graft patency in our cohort. A high fibrinogen level was significantly associated with graft failure in women but not in men. Fibrinogen plays an important role in patients with PAD. In the CHS cohort, ankle-brachial index levels were inversely correlated with fibrinogen levels.[37] Plasma fibrinogen has also been shown to be a predictor of the development[22,23] and severity of PAD.[17,21] In studies in PAD patients, fibrinogen levels were found to be the strongest independent predictor of death from coronary disease[38] and all-cause cardiovascular mortality.[39] There are fewer data reporting the association between fibrinogen level and outcomes after lower extremity revascularization. Two reports evaluating patients with lower extremity vein grafts demonstrated elevated fibrinogen levels were associated with vein graft stenoses[24] and graft occlusion.[25] In contrast, a study that evaluated 57 patients undergoing infrainguinal arterial reconstruction with saphenous vein narrowly failed to demonstrate a statistically significant association between fibrinogen levels and graft failure.[40] It is possible that a type II error occurred or, perhaps, that an unidentified interaction contributed to the overall negative results of the study. To our knowledge, no studies have evaluated the association between fibrinogen and LEB graft patency separately for women and men.

There are several studies that suggest that women have higher rates of graft failure and increased rates of wound complications after LEB graft procedures, but clear predisposing factors have not been identified.[5-7] It is possible that women who present with advanced PAD have an underlying inflammatory phenotype that puts them at a disproportionately higher risk for subsequent graft failure compared with men. Measurement of both CRP and plasma fibrinogen in women before revascularization may identify those who are at highest risk for graft failure and could potentially improve results either by aggressive risk factor modification or a more intensive graft surveillance schedule. Prospective studies are needed to validate the use of these biomarkers for risk prediction in this population.

This study has several limitations. Our series of patients had advanced PAD, including a large proportion with critical limb ischemia. Also, all patients were treated with a vein conduit. Therefore, our results may not be generalizable to those with more mild disease or in those who undergo LEB with a prosthetic graft. We dichotomized CRP at 5 mg/L and fibrinogen at 600 mg/dL, either or both of which may not be the ideal cut points. CRP and fibrinogen were not included together in the same multivariable Cox model, and hence we could not assess whether each biomarker was independently associated with graft failure. In addition, only preoperative levels of inflammatory markers were available in this study, and hence we could not evaluate whether changes in CRP and fibrinogen levels after LEB were associated with graft failure. The modest size and diversity of the study population also limit its generalizability. The findings should be considered hypothesis-generating and provide impetus for further studies examining the interaction of gender and inflammation in peripheral revascularization.

CONCLUSIONS

Women undergoing LEB for advanced PAD have a different inflammatory phenotype than men. Elevated baseline levels of CRP and fibrinogen are associated with inferior vein graft patency in women but not in men. These findings indicate an important interaction between sex and inflammation in the healing response of vein grafts for LEB. Women with elevated preoperative CRP and fibrinogen levels may benefit from more intensive postoperative graft surveillance protocols.

AUTHOR CONTRIBUTIONS

Conception and design: JH, CO, MB, MAC, MSC
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DISCUSSION

Dr Michelle Mueller (*Salt Lake City, Utah*). I would like to thank the program committee for inviting me to be a discussant. Congratulations to Dr Hiramoto on a very nice presentation and the authors on an excellent study and paper.

Many of us have the impressing that women with peripheral artery disease do more poorly than men. The question is why, and can we do anything to improve their surgical outcomes. In this prospective study, the authors show us that high-sensitivity C-reactive protein (CRP) and fibrinogen are not only elevated in women at baseline when compared to men, but those women with elevated CRP or fibrinogen have decreased primary graft patency.

A few questions and comments:

In the paper you state there are no differences in the outflow vessels (popliteal versus tibial versus pedal). However, there were significantly more women with critical limb ischemia than men. Is there a trend towards more infrapopliteal bypasses in women in general, and more specifically in those with elevated inflammatory markers?

Dr Jade S. Hiramoto. Thank you for reviewing our manuscript and for your kind comments. Indeed, there were significantly more women with critical limb ischemia in this cohort compared to men. However, there were no significant differences in the target outflow vessels by sex. In both men and women, about half underwent a bypass graft to the popliteal artery, and the other half underwent a bypass graft to either a tibial or pedal vessel. There was no statistically significant difference, nor was there a trend toward more infrapopliteal bypasses in women. Those patients with either CRP >5 mg/L or fibrinogen >600 mg/dL were more likely to undergo an infrapopliteal bypass compared to those with CRP <5 mg/L or fibrinogen <600 mg/dL, but this was true for both the men and women in this cohort.

Dr Mueller. Does the decreased primary graft patency in women with elevated CRP or fibrinogen correlate with graft thrombosis or limb loss, or are there just more interventions on grafts in these groups?

Dr Hiramoto. The discussant raises an important question. Of the 78 primary patency events, 26 (33%) were due to graft thrombosis and 52 (67%) were revisions for graft stenosis. Both men and women had similar profiles for loss of primary patency—37% of the primary patency events were graft thrombosis in women, and 31% were graft thrombosis events in men. There was no significant difference in the type of primary patency event (thrombosis or stenosis) based on CRP or fibrinogen values. This was true when analyzed in the overall cohort and when separately analyzed by sex.

We also analyzed secondary graft patency in this cohort and the findings were similar to those for primary graft patency. Women with CRP >5 mg/L were significantly more likely to lose secondary graft patency compared to women with CRP <5 mg/L, and women with fibrinogen >600 mg/dL were significantly more likely to lose secondary graft patency compared to women with fibrinogen <600 mg/dL. These findings were not seen in the men in this cohort.

Dr Mueller. Would a more intensive graft surveillance program decrease graft thrombosis and improve limb salvage?

Dr Hiramoto. It is difficult to know whether a more intensive graft surveillance program would decrease the graft thrombosis rate. Most of the losses of primary graft patency occurred within the first year, and we performed duplex ultrasound examination of the bypass grafts at 1, 3, 6, 9, and 12 months postoperatively. Based on the findings in this study, one might consider increasing the frequency of this surveillance in a higher risk patient, for instance, a woman with CRP >5 mg/L or fibrinogen >600 mg/dL. However, it is unclear whether this would change the overall outcome.

Dr Mueller. Finally, you recommend aggressive risk factor modification. Would you delay bypass in women with critical limb ischemia and elevated inflammatory markers for risk factor modification, and what modifications would you recommend? With our current knowledge regarding these inflammatory markers, are we not already encouraging smoking cessation and aggressively treating patients with statins? Perhaps we are failing to recognize peripheral artery disease early in women and also failing to aggressively medically mange them.

Dr Hiramoto. Because of the overall size of the cohort and relatively small numbers of women, I think that one should consider the findings from our study hypothesis-generating. I think it provides important background information for future studies to further evaluate this interaction between sex and inflammation. We believe that all at-risk individuals should stop smoking and be treated with statins, yet 37% of our cohort were still smoking, and 30% of the women in this cohort were not on statin therapy. So, there is still room for improvement. At this point in time, I would not recommend delaying bypass grafting in women with critical limb ischemia and elevated inflammatory markers for risk factor modification without further confirmatory data. Finally, I agree with Dr. Mueller—epidemiologic studies have demonstrated that peripheral artery disease prevalence is equal (if not higher) in women compared to men. We are not doing a good enough job at recognizing peripheral artery disease early in women, and hence, we are failing to provide the appropriate aggressive medical management.