An integrated biochemical prediction model of all-cause mortality in patients undergoing lower extremity bypass surgery for advanced peripheral artery disease

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Background: Patients with advanced peripheral artery disease (PAD) have a high prevalence of cardiovascular (CV) risk factors and shortened life expectancy. However, CV risk factors poorly predict midterm (<5 years) mortality in this population. This study tested the hypothesis that baseline biochemical parameters would add clinically meaningful predictive information in patients undergoing lower extremity bypass operations.
Methods: This was a prospective cohort study of patients with clinically advanced PAD undergoing lower extremity bypass surgery. The Cox proportional hazard model was used to assess the main outcome of all-cause mortality. A clinical model was constructed with known CV risk factors, and the incremental value of the addition of clinical chemistry, lipid assessment, and a panel of 11 inflammatory parameters was investigated using the C statistic, the integrated discrimination improvement index, and Akaike information criterion.
Results: The study monitored 225 patients for a median of 893 days (interquartile range, 539-1315 days). In this study, 50 patients (22.22%) died during the follow-up period. By life-table analysis (expressed as percent surviving ± standard error), survival at 1, 2, 3, 4, and 5 years, respectively, was 90.5% ± 1.9%, 83.4% ± 2.5%, 77.5% ± 3.1%, 71.0% ± 3.8%, and 65.3% ± 6.5%. Compared with survivors, decedents were older, diabetic, had extant coronary artery disease, and were more likely to present with critical limb ischemia as their indication for bypass surgery ($P < .05$). After adjustment for the above, clinical chemistry and inflammatory parameters significant (hazard ratio [95% confidence interval]) for all-cause mortality were albumin (0.43 [0.26-0.71]; $P = .001$), estimated glomerular filtration rate (0.98 [0.97-0.99]; $P = .023$), high-sensitivity C-reactive protein (hsCRP; 3.21 [1.21-8.55]; $P = .019$), and soluble vascular cell adhesion molecule (1.74 [1.04-2.91]; $P = .034$). Of the inflammatory molecules investigated, hsCRP proved most robust and representative of the integrated inflammatory response. Albumin, eGFR, and hsCRP improved the C statistic and integrated discrimination improvement index beyond that of the clinical model and produced a final C statistic of 0.82.
Conclusions: A risk prediction model including traditional risk factors and parameters of inflammation, renal function, and nutrition had excellent discriminatory ability in predicting all-cause mortality in patients with clinically advanced PAD undergoing bypass surgery. (J Vasc Surg 2012;56:686-95.)

Patients undergoing arterial reconstructive procedures for advanced peripheral artery disease (PAD) tend to be elderly and have a high prevalence of cardiovascular risk factors, including diabetes mellitus, hypertension, dyslipidemia, and tobacco abuse. Collectively, these risk factors are known to predict long-term mortality (≥10 years) but are not sensitive at identifying patients at risk for death in the near-term or midterm (≤5 years).[1] Recently developed risk prediction models and scoring systems of 1-year or 2-year mortality in patients after lower extremity bypass surgery have revealed that patient-specific risk factors, such as inadequate saphenous vein, the presence of critical limb ischemia, advanced age, or the Bollinger below-knee angiogram score, are the most potent independent predictors.[2-4] Although these unique risk factors are unlikely to be responsible for death per se, they are most likely surrogates of the patient's overall frailty.

Patients with PAD are known to have increased levels of inflammatory cytokines,[5] acute-phase reactants, and soluble adhesion molecules, even after adjusting for age, sex, and traditional risk factors.[6] Elderly individuals with the highest levels of inflammatory biomarkers have increased short-term mortality (<5 years).[7,8] Inflammation in the PAD population, as assessed by high-sensitivity C-reactive protein (hsCRP), not only correlates directly with severity of PAD, which is higher in patients with critical limb

ischemia (CLI), but is also associated with death and cardiovascular events.[9-11]

The hypothesis of this study was that increased baseline inflammation would add clinically meaningful value to predicting all-cause mortality in patients undergoing lower extremity bypass surgery. We a priori chose to examine a panel of 11 novel and diverse inflammatory biomarkers based on epidemiologic evidence implicating them with adverse cardiovascular outcomes and death. We sought to determine which biomarkers among these, if any, add predictive value beyond that of pre-existing established risk factors for patients undergoing lower extremity bypass surgery.

METHODS

Study design and population. This was a National Institutes of Health–funded prospective cohort study examining the relationship between inflammatory biomarkers and death after lower extremity bypass surgery. Three participating institutions underwent independent review of the study and received approval from the respective Institutional Review Boards. Each patient provided written informed consent. Enrollment began in February 2004 and ended in May 2008.

Details of patient selection and inclusion and exclusion criteria have been published previously.[10,12,13] Patients were invited to participate in this study if they planned to undergo a lower extremity bypass operation for disabling claudication or critical limb ischemia due to atherosclerotic obstructive disease.

Because the hypothesis to be tested was that the baseline inflammatory and biochemical profile was predictive of death, we excluded anyone with active infection or concurrent illness that would cause a spurious increase in the plasma concentration of inflammatory biomarkers beyond the baseline state. Therefore, our exclusion criteria included any evidence of active infection, pneumonia, malignancy, autoimmune disorders, or other precedent or concurrent significant illness 30 days before the index bypass procedure. Although the study accepted patients with small ulcers or small areas of dry gangrene, patients with deep space infections of the foot, those with large areas of ulceration, osteomyelitis, or ulceration or gangrene requiring operative debridement were excluded from participation. Also excluded were patients taking immunosuppressive medications or oral steroids. Because another aim of the present protocol involved the analysis of inflammation on vein graft-specific outcomes, only patients undergoing a bypass with autogenous vein were consented. The study enrolled 225 patients.

Blood processing and assay measurements of biomarkers. Plasma was collected with the patient in the fasting state on the morning of the bypass procedure. Blood was collected into ethylenediaminetetraacetic acid and citrate Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) and immediately iced. Tubes were spun at 3000 rpm for 20 minutes at 4°C in a refrigerated centrifuge. All samples were stored at −80°C until analysis. All analyses were conducted in batch at a core laboratory to avoid variation.

The Modification of Diet in Renal Disease Study equation was used to estimate glomerular filtration rate (eGFR) from serum creatinine (SCr): eGFR (mL/min/1.73 m²) = $175 \times (SCr)^{-1.15} \times (age)^{-0.203} \times (0.742$ if female$) \times (1.210$ if black$)$.[14] Body mass index (BMI) was expressed as kg/m².

Clinical and end point definitions. The end point investigated in this study was all-cause mortality (death). Race, ethnicity, and sex were assessed by self-report. Patients were considered to have hypertension if they were taking prescription medications for hypertension or if their systolic blood pressure was >140 mm Hg or diastolic blood pressure was >90 mm Hg. A diagnosis of hypercholesterolemia was present if they were taking prescription medications for cholesterol or if they self-reported a prior diagnosis. Patients were considered to have diabetes mellitus if they were taking prescription medications (oral or insulin) for diabetes or if they self-reported a prior diagnosis. Patients were considered to have coronary artery disease (CAD) if they had stable angina, prior myocardial infarction, a prior coronary revascularization procedure, positive result on a stress test, abnormal result on a coronary angiogram, or ischemic cardiomyopathy. Active smoking was determined by self-report. Former smokers were defined as individuals who had smoked >100 cigarettes in their life but had not smoked in the past 30 days, and never-smokers were defined as individuals who had never smoked.

Statistical analysis. Baseline characteristics and biomarker values are presented as mean ± standard deviation or median (interquartile range [IQR]), depending on the normality of their distribution. Proportions between groups were compared with χ^2 test. Correlations were assessed with Pearson correlation coefficients on lognormalized marker values. Univariate differences in plasma levels of markers between subgroups of patients were analyzed with one-way nonparametric Wilcoxon rank sum (Mann-Whitney *U*) tests. Standard univariate analysis was performed between patient demographics, laboratory and lipid values, and inflammatory biomarkers and the primary outcome of death by log-rank test. Inflammatory biomarkers were expressed as tertiles, with the lowest tertile being the referent. In a preliminary analysis, we explored multiple ways to express inflammatory markers, and dividing them into tertiles was most representative of discriminating mortality. The exception to this was hsCRP, which we modeled as a dichotomized variable as we have done in the past.[10,12]

Multivariable Cox proportional hazard modeling was performed on survival outcomes, and hazard ratios (HR) and 95% confidence intervals (CI) are presented. A test of the proportional hazard assumption was performed for each covariate and globally using a formal significance test based on the unscaled and scaled Schoenfeld residuals. In the model construction, we followed the natural hierarchy of decision making for the patient with PAD. First, a clinical model was constructed from all readily available clinical variables, including demographic data, medical history, and

Table I. Demographic characteristics of the study population

Demographics[a]	Overall (N = 225)	Alive (n = 175)	Dead (n = 50)	P
Age, years	67.62 ± 10.93	66.47 ± 10.75	71.66 ± 10.69	.0029
Male sex	161 (71.56)	127 (72.57)	34 (68.0)	.527
BMI, kg/m^2	28.36 ± 6.51	28.27 ± 6.18	28.70 ± 7.64	.6804
Hypertension	190 (84.4)	146 (83.43)	44 (88.00)	.432
CAD	118 (52.4)	85 (48.57)	33 (66.00)	.030
Hyperlipidemia	162 (72.0)	125 (71.43)	37 (74.00)	.721
White race	196 (87.1)	149 (85.14)	47 (94.00)	.099
CLI	133 (59.1)	93 (53.14)	40 (80.00)	.001
Diabetes	117 (52.0)	85 (48.57)	32 (64.00)	.054
Tobacco use				
Former	98 (43.6)	77 (44.00)	21 (42.00)	.801
Current	83 (36.9)	65 (37.14)	18 (36.00)	.883
Medication use				
Statin	180 (80)	137 (78.3)	43 (86.00)	.217
Antiplatelet	182 (81)	142 (81.14)	40 (80.00)	.545
ACEI/ARB	126 (56)	104 (59.43)	22 (48.00)	.175
β-Blocker	183 (81.3)	141 (80.57)	42 (84.00)	.993

ACEI, Angiotensin-converting enzyme inhibitor; *ARB,* angiotensin receptor blocker; *BMI,* body mass index; *CAD,* coronary artery disease; *CLI,* critical limb ischemia.
[a]Continuous variables are shown as mean ± standard deviation, and categoric variables as number (%).

Table II. Clinical chemistry and lipid biomarker values

Biomarker[a]	Overall (N = 225)	Claudication (n = 92)	CLI (n = 133)	P[b]	Alive	Dead	P[b]
Albumin, g/dL	3.60 ± 0.51	3.73 ± 0.41	3.52 ± 0.54	.0071	3.67 ± 0.46	3.37 ± 0.60	.0009
Hemoglobin, g/dL	11.95 ± 1.80	12.72 ± 1.72	11.42 ± 1.65	<.00001	12.12 ± 1.78	11.36 ± 1.75	.0077
WBC, ×10^3/mm^3	7.90 ± 2.57	7,83 ± 2.30	7.94 ± 2.75	.7425	7.87 ± 2.68	7.99 ± 2.18	.7656
Calcium, mg/dL	8.64 ± 0.54	8.65 ± 0.51	8.63 ± 0.56	.7987	8.65 ± 0.54	8.60 ± 0.54	.5336
PO$_4$, mg/dL	3.49 ± 1.03	3.35 ± 0.67	3.58 ± 1.22	.2170	3.43 ± 0.90	3.69 ± 1.40	.2350
eGFR, mL/min/1.73 m^2	76.05 ± 34.87	85.76 ± 27.95	69.29 ± 37.61	.0004	80.80 ± 33.17	59.52 ± 35.89	.0001
Cholesterol, mg/dL							
Total	128.10 ± 35.74	138.93 ± 38.53	120.87 ± 31.90	.0003	132.25 ± 35.63	113.13 ± 32.23	.0012
LDL	63.74 ± 25.68	70.50 ± 25.34	59.49 ± 25.08	.0025	66.46 ± 26.07	54.23 ± 22.00	.0042
HDL	34.36 ± 10.60	35.01 ± 9.73	33.92 ± 11.16	.4675	34.71 ± 9.99	33.08 ± 12.59	.3574
ApoA1, mg/dL	95.42 ± 24.11	100.71 ± 23.53	93.44 ± 24.16	.0289	98.92 ± 22.78	87.80 ± 26.86	.0050
ApoB-100, mg/dL	57.72 ± 17.81	63.30 ± 17.32	53.71 ± 17.12	.0001	59.78 ± 17.63	50.36 ± 16.62	.0012
Lp(a), mg/dL	33.10 ± 32.86	33.79 ± 33.98	32.60 ± 32.15	.7949	35.24 ± 33.65	25.43 ± 28.87	.0702
Triglycerides, mg/dL	152.49 ± 130.14	165.60 ± 154.54	143.74 ± 110.76	.2339	158.64 ± 143.88	130.54 ± 55.17	.1963

Apo, Apolipoprotein; *CLI,* critical limb ischemia; *eGFR,* estimated glomerular filtration rate; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *Lp(a),* lipoprotein a; *PO$_4$,* phosphate; *WBC,* white blood cells.
[a]Variables are expressed as mean ± standard deviation.
[b]*P* value obtained through the Student *t*-test or χ^2 test, where appropriate.

disease severity on presentation (ie, CLI or claudication). This was noted as the clinical model, and entry into the clinical model required a univariate value of $P < .10$. We next determined the added benefit of clinical chemistry, lipid concentrations, and inflammatory parameters conditional on the clinical model.

Owing to the number of inflammatory biomarkers examined, we broadly categorized this panel into three groups, recognizing that there is considerable overlap among them. The three groups included:

- The coagulation and fibrinolytic system, including plasminogen activator inhibitor type 1, tissue plasminogen activator, and fibrinogen;
- The soluble (s) adhesion molecules and receptors, including vascular cell adhesion molecule (sVCAM-1), intercellular adhesion molecule 1 (sICAM-1), sP-selectin, and tumor necrosis factor (TNF) receptor type 2 (sTNFR2), which was used as a proxy for TNF-α because it has been shown to be highly correlated with TNF-α, is more stable in plasma, and is more relevant for cardiovascular-related outcomes; and
- The inflammatory cytokines and pentraxins, including interleukin (IL)-6, monocyte chemotactic protein-1, hsCRP, and serum amyloid A.

Entry inclusion into the model for these parameters required a univariate $P < .10$ and a Harrell C statistic > .65. This cutoff allowed retention of a diverse set of clinical and inflammatory parameters that we concluded adequately represented the patient's biochemistry.

The ability to classify risk was assessed by the use of the Harrell C statistic.[15] To determine the degree of risk reclassification, the integrated discrimination improvement index (IDI) was used. The IDI can be thought of as indicating how far individuals are moving on average along the continuum of predicted risk; that is, how well a model separates individuals by the outcome of interest (death) by estimating the change in the difference in the mean predicted probabilities of the outcome between those with and without the outcome in question after introducing the candidate biomarker to the model.[16] Finally, we sought to obtain the most parsimonious model by minimizing the Akaike information criterion (AIC), which is a metric of the goodness-of-fit and accounts for the number of parameters required to obtain that degree of fitness. Statistical analyses were performed on Stata/SE 11.0 software (StataCorp LP, College Station, Tex).

RESULTS

Population demographics. The demographic summary of the study population is presented in Table I. The indication for bypass surgery was CLI in 133 patients (59.1%), classified as Fontaine 2b in 92, Fontaine 3 in 60, and Fontaine 4 in 73. The mean follow-up from the index bypass procedure was 925 days (median, 893; IQR, 539-1315 days). No surviving individual was monitored <365 days. Fifty patients (22.2%) died during the follow-up period. By life-table analysis (expressed as percentage surviving ± standard error), survival at 1, 2, 3, 4, and 5 years, respectively, was 90.5% ± 1.9%, 83.4% ± 2.5%, 77.5% ± 3.1%, 71.0% ± 3.8%, and 65.3% ± 6.5%. Two deaths occurred ≤30 days and 28 deaths ≤1 year. Compared with survivors, decedents were older (mean age, 71.7 ± 10.7 vs 66.5 ± 10.8 years; P = .003), had a higher incidence of established CAD (66% vs 49%; P = .030), and were more likely to present with CLI as their indication for bypass surgery (80% vs 53.1%; P = .001; Table I). The use of a statin at the time of bypass was not associated with survival; however, only 45 patients (20%) were not taking a statin and only seven deaths occurred among these individuals during follow-up.

The bypass graft characteristics and outcomes have been published elsewhere.[10,12,13] In brief, 202 bypass grafts were constructed from a single segment or composite great saphenous vein and 23 were from an arm vein. There were 111 femoral-popliteal, 78 femoral-tibial, and 36 femoral or popliteal-pedal bypass grafts. The 2-year primary and secondary patency rates were 61.4% ± 3.6% and 83.1% ± 2.7%, respectively.

Concentrations of albumin, hemoglobin, and eGFR were all significantly lower in the CLI subgroup (Table II). As expected, most biomarkers of inflammation were higher in patients who presented with CLI compared with those presenting with claudication (Table III). However, there was no difference in the white blood cell count (Table II). The finding of no difference in white blood cell count between CLI and claudication patients was expected given our stringent exclusion criteria for entry into this study.

Total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein (apo) A1 and apoB100 were lower in patients presenting with CLI (Table II).

Biomarkers and death. Baseline albumin, hemoglobin, eGFR, and total cholesterol and LDL cholesterol concentrations were lower in decedents than in survivors (log-rank P < .05; Table II). In particular, albumin (3.37 ± 0.60 vs 3.67 ± 0.46 mg/dL; P = .0009) and eGFR (59.5 ± 35.9 vs 80.8 ± 33.2 mL/min/1.73 m^2; P = .0001) were significantly lower in patients who subsequently died compared with those who survived. By contrast, baseline levels of all inflammatory cytokines and pentraxins, fibrinogen, and all soluble receptors and cell adhesion molecules, except sP-selectin, were markedly elevated in patients who subsequently died (Table III). All biomarkers were positively and significantly correlated with one another and negatively correlated with albumin (P < .0002 for all correlations). The Pearson correlation coefficient between albumin and total cholesterol was .27 (P = .0006), reflecting the nutritionally depleted state in patients who subsequently died during follow-up. However, there was no correlation between albumin and the patient's BMI (P = .07).

Table IV summarizes the univariate association expressed as HR (95% CI) and as the C statistic between patient demographics and laboratory and lipid values with all-cause mortality. All biomarkers were modeled as tertiles with the exception of CRP. The biomarker CRP was modeled as a dichotomized variable with a cutoff of 5 mg/L. We chose to do this because our previous work has shown that CRP offers the best ability to discriminate between patients undergoing lower extremity bypass surgery into those with and without adverse graft-related events.[10] Further, patients with CRP levels >5 mg/L exhibit impaired vein graft remodeling compared with patients with lower CRP levels, implying that this value is clinically relevant in vein graft-related outcomes.[12] In addition, hsCRP >5 mg/L had a sensitivity of 70.2% and specificity of 72.0% for subsequent midterm mortality. This represents the highest sum of sensitivity and specificity and therefore is an optimal cutoff for this analyte. By univariate analysis, an hsCRP level >5 mg/L was associated with increased mortality (HR, 4.54; 95% CI, 2.43-8.49; P < .0001; Fig 1).

In multivariable analysis, clinical risk factors that met the inclusion criteria included age, diabetes mellitus, the presence of established CAD, and CLI as an indication for bypass. Clinical and inflammatory biomarker values that met the inclusion criteria included albumin, eGFR, hsCRP, sVCAM-1, sTNFR2, and fibrinogen (Table V). Representative biomarkers from each of the three broad categories were present in the final model. Although IL-6 formally met the inclusion criteria into the final model, it was omitted given its known role of induction of CRP production.[17] Lower tertiles of total cholesterol, LDL, high-density lipoprotein, and apoA1 and apoB100 were associated with decreased survival on univariate analysis; however, no lipid parameter met the inclusion criteria for the final model. The HRs and 95% CI of all variables entered into the final model are presented in Table V.

Table III. Inflammatory marker values broadly grouped into coagulation and fibrinolytic factors, soluble cell adhesion molecules and receptors, and inflammatory cytokines and pentraxins

Biomarker[a]	Overall (N = 225)	Claudication (n = 92)	CLI (n = 133)	P[b]	Alive	Dead	P[b]
Coagulation and fibrinolytic factors							
PAI-1, ng/mL	52.78 (31.84-87.83)	50.33 (28.07-88.84)	53.46 (32.89-86.38)	.6561	51.89 (30.44-85.46)	57.96 (34.01-111.16)	.1990
tPA, ng/mL	9.95 (5.39-14.03)	10.03 (5.39-13.25)	9.70 (5.63-15.19)	.8292	9.77 (5.36-13.96)	10.25 (5.89-15.44)	.4936
Fibrinogen, mg/dL	479.95 (401.00-585.00)	438.60 (381.30-504.50)	521.30 (447.20-624.30)	.0001	463.05 (390.85-545.30)	587.35 (473.80-673.80)	.0001
Soluble cell adhesion molecules and receptors							
P-selectin, ng/mL	51.12 (38.05-69.38)	49.04 (37.34-68.79)	51.89 (38.39-70.67)	.5173	51.51 (38.18-70.41)	50.74 (37.93-66.38)	.6949
VCAM-1, ng/mL	706.40 (536.75-1079.55)	604.90 (462.80-836.40)	801.3 (596.8-1310.45)	.0001	655.90 (505.90-944.60)	1048.70 (682-80-1496.0)	.0001
ICAM, ng/mL	261.35 (218.65-322.55)	247.50 (212.40-315.10)	267.85 (220.95-338.75)	.0956	251.60 (215.50-315.20)	288.00 (244.20-360.80)	.0071
sTNFR2, ng/mL	2.80 (2.12-4.32)	2.28 (1.89-3.09)	3.41 (2.48-5.34)	.0001	2.65 (2.02-3.81)	3.81 (2.78-8.07)	.0001
Inflammatory cytokines and pentraxins							
IL-6, pg/mL	4.74 (2.01-10.22)	2.78 (1.50-5.22)	6.67 (3.25-12.83)	.0001	3.63 (1.58-8.12)	10.97 (4.70-23.68)	.0001
CRP, mg/L	2.98 (1.28-12.25)	1.81 (0.92-3.95)	5.53 (1.68-21.68)	.0001	2.39 (1.20-5.58)	13.97 (2.94-41.19)	.0001
SAA, mg/dL	0.86 (0.42-2.09)	0.62 (0.32-0.93)	1.33 (0.56-4.5)	.0001	0.77 (0.41-1.48)	2.14 (0.64-9.26)	.0001
MCP-1, pg/mL	215.65 (176.90-278.50)	196.10 (168.30-248.90)	229.70 (180.80-308.40)	.0014	208.50 (174.00-265.00)	253.60 (181.10-357.40)	.0100

CLI, Critical limb ischemia; *CRP,* C-reactive protein; *ICAM,* intercellular adhesion molecule; *IL-6,* interleukin 6; *MCP-1,* monocyte chemotactant protein; *PAI-1* plasminogen activator inhibitor type 1; *SAA,* serum amyloid A; *sTNFR2,* soluble tumor necrosis factor receptor 2; *tPA,* tissue plasminogen activator; *VCAM-1,* vascular cell adhesion molecule.
[a]Values are presented as median (interquartile range).
[b]Kruskal-Wallis rank test.

Higher eGFR (HR, 0.98; 95% CI, 0.97-0.99; $P = .023$) and albumin (HR, 0.43; 95% CI, 0.26-0.71; $P = .001$) were protective for overall death, whereas higher hsCRP >5 mg/L (HR, 3.21; 95% CI, 1.21-8.55; $P = .019$) and sVCAM-1 (HR, 1.74; 95% CI, 1.04-2.91; $P = .034$) were predictive of death. The hazard of reduced serum albumin was linear through the full range of albumin (2.2 to 4.9 mg/dL). In addition, albumin predicted death equally well for patients with high levels of inflammatory biomarkers, assessed as hsCRP >5 mg/L (HR, 0.48; 95% CI, 0.28-0.83), as it did for patients with hsCRP <5 mg/L (HR, 0.49; 95% CI, 0.29-0.84), indicating an effect size that was independent of the patient's inflammatory state. Although the HR for each unit increase of eGFR was marginal at 0.98, the hazard for reduced renal function begins well before the onset of hemodialysis.[18] In the present analysis, each standard deviation 35 mL/min/1.73 m²–increase in eGFR had an HR of 0.54.

The clinical model produced a C statistic of 0.71 (Table VI). When eGFR, albumin, and hsCRP were added to the model, the C statistic was increased to 0.82. Among all of the biochemical parameters investigated, albumin contributed the largest increase in the C statistic (0.09) beyond the clinical model. The addition of hsCRP to the clinical model plus eGFR and albumin improved the C statistic by 0.02. The IDI, an assessment of incremental improvement in risk discrimination after addition of a new predictor, was estimated for the addition of eGFR, albumin, and hsCRP to the clinical model, and all were highly significant (Table VI). The addition of other clinical or inflammatory markers (sVCAM, sTNFR2) failed to significantly improve the discriminatory ability of the final model, which consisted of age, diabetes, CAD, CLI, albumin, eGFR, and hsCRP.

DISCUSSION

Management decisions for patients with severe PAD are based on multiple levels of information processed in a natural hierarchical order; for example, demographic characteristics, prevalent and medical history, and clinical presentation are considered first, followed by results of routine laboratory and angiographic tests. The addition of a biomarker must be predicated on its ability to provide clinically meaningful information above that provided by routine history, examination, and laboratory tests.

Table IV. Univariate association of demographic, clinical, and lipid parameters with all-cause mortality

| Predictor variables	HR (95% CI) for death	P	C statistic
Baseline demographics and disease presentation			
Age	1.04 (1.01-1.07)	.003	0.6288
Male sex	0.85 (0.46-1.54)	.598	0.5146
White race	0.62 (0.29-1.32)	.224	0.5335
Diabetes mellitus	1.69 (0.95-3.01)	.074	0.5618
CAD	1.96 (1.09-3.53)	.023	0.6002
Hypertension	1.44 (0.61-3.38)	.401	0.5152
Hyperlipidemia	1.08 (0.57-2.04)	.791	0.5114
Tobacco (current)	0.77 (0.43-1.37)	.379	0.5352
CLI	3.35 (1.67-6.71)	.001	0.6358
Clinical laboratory values			
Albumin[a]	0.37 (0.24-0.58)	<.0001	0.7049
Hemoglobin[a]	0.53 (0.37-0.78)	.001	0.6213
WBC[a]	1.13 (0.79-1.62)	.479	0.5397
Calcium	0.73 (0.43-1.23)	.242	0.5655
Phosphate	1.04 (0.75-1.45)	.789	0.4297
eGFR	0.98 (0.97-0.99)	<.0001	0.6503
Lipid values			
Total cholesterol[a]	0.62 (0.43-0.91)	.014	0.6218
LDL[a]	0.59 (0.40-0.86)	.007	0.6415
HDL[a]	0.63 (0.43-0.92)	.018	0.6178
Apolipoprotein A1[a]	0.66 (0.45-0.96)	.034	0.5984
Apolipoprotein B100[a]	0.62 (0.43-0.90)	.013	0.6158
Lp(a)[a]	0.84 (0.59-1.19)	.351	0.5386
Triglycerides[a]	1.06 (0.75-1.50)	.727	0.4982
Fibrinolytic factors			
PAI-1	1.19 (0.83-1.70)	.332	0.5395
tPA	1.04 (0.73-1.49)	.792	0.5305
Fibrinogen	2.23 (1.50-3.33)	<.0001	0.6810
Soluble cell adhesion molecules and receptors			
sP-selectin	0.93 (0.65-1.32)	.705	0.5061
sVCAM-1	2.15 (1.45-3.20)	<.0001	0.6824
sICAM-1	1.67 (1.16-2.42)	.006	0.6452
sTNFR2	1.93 (1.32-2.83)	.001	0.6572
Inflammatory cytokines and pentraxins			
Interleukin-6	2.37 (1.58-3.56)	<.0001	0.6943
hsCRP >5 mg/L	4.54 (2.43-8.49)	<.0001	0.7001
MCP-1	1.43 (1.00-2.06)	.049	0.5759
SAA	1.90 (1.29-2.81)	.001	0.6453

CAD, Coronary artery disease; *CI,* confidence interval; *CLI,* critical limb ischemia; *eGFR,* estimated glomerular filtration rate; *HDL,* high-density lipoprotein; *HR,* hazard ratio; *hsCRP,* high-sensitivity C-reactive protein; *LDL,* low-density lipoprotein; *Lp(a),* lipoprotein (a); *MCP-1,* monocyte chemotactant protein; *PAI-1* plasminogen activator inhibitor type 1; *SAA,* serum amyloid A; *sICAM-1,* soluble ICAM, intercellular adhesion molecule; *sTNFR2,* soluble tumor necrosis factor receptor 2; *sVCAM-1,* soluble vascular cell adhesion molecule; *tPA,* tissue plasminogen activator; *WBC,* white blood cell.
[a]Biomarker was modeled as a tertile.

For any risk model to have practical value, it must be intuitive and easy to incorporate into clinical practice. The final model constructed in this study contains a diverse set of variables, including traditional demographics, such as age and diabetes, severity of PAD, a measure of renal function, an assessment of protein-energy malnutrition, and a marker of inflammation. The seven parameters represented in the final model are naturally intuitive and readily obtained in a physician's office; hence, its practical value. In addition, the final model has a C statistic of 0.82, which is within the range considered to have excellent discriminatory ability.[19] A C statistic of 0.82 indicates that the model can adequately discriminate between a randomly selected patient who dies during follow-up and a randomly selected patient who survives, 82% of the time. Finally, the model minimizes the AIC, a metric of goodness-of-fit and complexity, because it imposes a penalty for the number of parameters needed to achieve the particular degree of fitness. It is a quantification of model preference seeking the fewest explanatory variables required to provide adequate fit to the data.

One of the immediate observations of the multivariable model is that traditional risk factors, including cholesterol fractions and smoking, were not significantly associated with the primary end point of midterm death in this cohort. Rather, impaired renal function, malnutrition, and inflammation were the most powerful predictors. This is consistent with previous investigations that have shown markers of inflammation are better predictors of short-term and midterm mortality, whereas traditional cardiovascular risk factors are better in the long-term (≥10 years).[7,11,20,21] Models incorporating more proximate causes of midterm death are desirable not only to inform management deci-
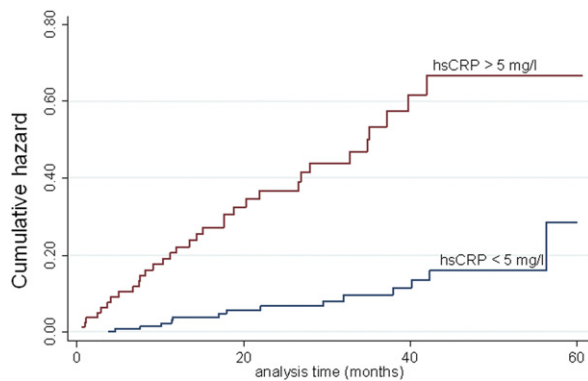
Fig 1. Nelson-Aalen cumulative hazard estimates for death are shown for patients dichotomized by preoperative baseline high-sensitivity C-reactive protein (*hsCRP*) values higher or lower than 5.0 mg/L.

Table V. Clinical and inflammatory biomarker values that met the inclusion criteria

Variable	Parameter estimate[a]	HR (95% CI)	P
Age	−0.003 ± 0.017	0.99 (0.96-1.03)	.859
Diabetes	0.125 ± 0.410	1.10 (0.50-2.41)	.807
CAD	0.490 ± 0.412	1.59 (0.72-3.52)	.249
CLI	0.614 ± 0.475	1.86 (0.73-4.73)	.192
eGFR	−0.013 ± 0.006	0.98 (0.97-0.99)	.023
hsCRP > 5 mg/L	1.15 ± 0.499	3.21 (1.21-8.55)	.019
Albumin (tertiles)	−0.835 ± 0.254	0.43 (0.26-0.71)	.001
sVCAM-1 (tertiles)	0.595 ± 0.300	1.74 (1.04-2.91)	.034
sTNFR2 (tertiles)	−0.101 ± 0.341	0.90 (0.46-1.76)	.767
Fibrinogen (tertiles)	−0.277 ± 0.327	0.75 (0.39-1.43)	.391

CAD, Coronary artery disease; *CI,* confidence interval; *CLI,* critical limb ischemia; *eGFR,* estimated glomerular filtration rate; *HR,* hazard ratio; *hsCRP,* high-sensitivity C-reactive protein; *sTNFR2,* soluble tumor necrosis factor receptor 2; *sVCAM-1,* soluble vascular cell adhesion molecule.
[a]Presented with the standard error.

sions but to allow for a potentially mitigating intervention. Identification of a high-risk patient may encourage the practitioner to choose a less invasive alternative to bypass such as endovascular revascularization. Understanding midterm survival seems especially prudent in light of the recently completed Bypass versus Angioplasty for Severe Ischaemia of the Leg (BASIL) trial demonstrating an overall survival advantage in patients randomized to bypass surgery in patients who survived at least 2 years.[2]

We felt it was unlikely that one marker could adequately represent the total inflammatory burden and that the simultaneous measurement of a panel of biomarkers would more adequately represent the integrated inflammatory risk. Thus, we measured 11 novel markers from three broad categories: thrombotic/fibrinolytic, soluble cell surface re-

Table VI. Model diagnostics: Cox proportional hazard model C statistic for all-cause midterm mortality, Akaike information criterion (*AIC*), and integrated improvement index (*IDI*)[a]

Risk markers	C statistic	AIC
Clinical model rf	0.7072	485.01
Clinical model rf + eGFR	0.7179	481.73
Clinical model rf + eGFR + albumin	0.7986	352.35
Clinical model rf + eGFR + albumin + hsCRP >5 mg/L	0.8200	325.00
Clinical model rf + eGFR + albumin + hsCRP > 5 mg/L + sVCAM-1	0.8190	320.78

| IDI	Estimate	P
IDI adding eGFR to clinical model rf	0.03358	.03284
IDI adding albumin to clinical model rf + eGFR	0.06786	.00048
IDI adding hsCRP to clinical model rf + eGFR + albumin	0.08352	.00005
IDI adding sVCAM to clinical model rf + eGFR + albumin + hsCRP >5 mg/L	0.01200	.17352[b]

eGFR, Estimated glomerular filtration rate; *hsCRP,* high-sensitivity C-reactive protein; *rf,* risk factor; *sTNFR2,* soluble tumor necrosis factor receptor 2; *sVCAM-1,* soluble vascular cell adhesion molecule.
[a]Clinical model rf includes age, diabetes mellitus, coronary artery disease, critical limb ischemia.
[b]P for the addition of sVCAM-1, sTNFR2, and fibrinogen is not significant.

ceptors and adhesion molecules, and inflammatory cytokines and acute-phase reactants. However, the addition of hsCRP to the full clinical model plus eGFR and albumin essentially quenched the model from further contribution of any other inflammatory biomarker. Although sVCAM retained statistical significance in the multivariable model, no additional clinically meaningful information, as determined by the C statistic or the IDI, was gained from its inclusion. In this regard, hsCRP has proved the most robust marker for inflammation in this cohort of patients undergoing lower extremity bypass surgery.

The inclusion of the full biomarker panel, however, is heuristic because enrolled patients, particularly those who died during follow-up, had a marked elevation in proinflammatory cytokines such as sTNFR2 and IL-6. These cytokines elicit an acute-phase response that has profound nutritional implications.[22] It is energy-intensive, with high rates of hepatic protein synthesis, and requires large quantities of essential amino acids. Through a variety of described pathways,[23] long-term exposure to elevated levels of cytokines enhances protein catabolism and induces anorexia, and lean muscle mass is expended to support synthesis of acute-phase proteins. The culmination of these processes creates a state of vascular cachexia. Indeed, the values of IL-6 and sTNFR2 in patients who died during follow-up were comparable to those in patients with advanced-stage congestive heart failure and those with known cardiac cachexia.[24,25] Similar to other forms of nonmalignant cachexia, this is a chronic hypercytokinemic

Malnutrition (albumin < 3.5mg/dl)   Renal impairment (eGFR <60 ml/min)
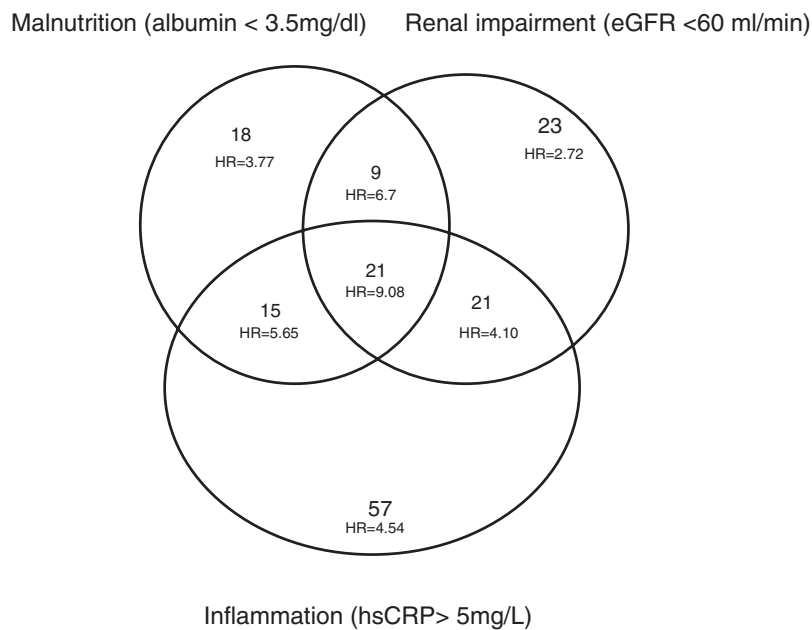
Fig 2. A Venn diagram demonstrates the overlap among subsets of patients with malnutrition, renal impairment, and inflammation and shows the synergism of different mechanisms for the hazard ratio (*HR*) of death. Of 225 patients in this study, 74 (33%) had an estimated glomerular filtration rate (*eGFR*) <60 mL/min/1.73 m^2, 63 (28%) had an albumin concentration of <3.5 mg/dL, and 80 (36%) had high-sensitivity C-reactive protein (*hsCRP*) concentration of >5.0 mg/L. The 21 patients (9%) located within the intersection of all three circles have an HR for death of 9.08.

cachexia, with relative preservation of body weight and BMI in most patients.[26] For example, only 32 patients in this study had a BMI <22 kg/m^2, and no correlation existed between BMI and albumin. Along with albumin, reduced hepatic production of lipoproteins is also associated with the inflammatory/malnourished state.[27] Cholesterol fractions and both apoA1 and apoB100 were decreased in patients who died in this study. However, the seemingly paradoxic finding that lower total cholesterol, high-density lipoprotein, LDL, apoA1, and apoB100 had significant univariate association with death was mitigated by inclusion of inflammatory, nutritional, and renal parameters. Therefore, in this study as in others, the so-called J-shaped association between lipid parameters and death is probably a result of confounding.[28]

The combined effect of inflammation, renal failure, and malnutrition has received little attention in patients undergoing lower extremity bypass despite the considerable overlap among them. Synergism of each to the hazard of death can be demonstrated by Venn diagrams, with relative malnourishment (albumin <3.5 mg/dL; n = 74), renal impairment (eGFR <60 mL/min; n = 63), and high levels of inflammation (hsCRP >5 mg/L; n = 80) represented (Fig 2). This is particularly alarming, because the 21 patients (9%) located at the convergence of all three circles had an HR of death of 9.08 (95% CI, 4.85-17.01); therefore, even modest impairment of more than one of these three domains has a substantial clinical consequence.

Limitations. Among the study imitations are that the cohort consisted predominantly of elderly white men. Further studies are needed to determine whether this model improves risk stratification in women, younger individuals, and other ethnic groups.

Some biomarker values in this study were extremely elevated. Because of the strict exclusion criteria applied and to the best of our knowledge, the elevated marker values likely reflect the true baseline state of the patient rather than spurious elevation due to concurrent illness. Albumin, which has a half-life of approximately 20 days, was used as a nutritional index in this examination. It is possible that another metric of nutrition would have been more appropriate, such as transthyretin (prealbumin; half-life of 2 days), which is more sensitive than albumin to changes in protein-energy status.[29] However, although transthyretin accurately reflects recent nitrogen intake, albumin may better represent the overall baseline nutritional state of the patient.[30] Finally, this study was modest in size and results must be considered with caution until external validation studies can be undertaken.

Study implications. Several methods, including the C statistic, IDI, and AIC, were used to provide diagnostic information about model iterations. Together these measures not only quantify calibration and discrimination but also ensure that the most parsimonious model is used to best fit the data. A model incorporating biochemical parameters has theoretic advantages to guide potential treatment strategies to improve survival among high-risk sub-

jects. Anti-inflammatory or anticytokine therapy may mitigate some of the effects of the acute-phase response seen in this population.[31,32] Nutritional therapy with ω-3 fatty acids, appetite stimulants, or metabolic regulators have shown promise in similar cohorts and may improve the recovery period after surgery and improve functional capacity.[33,34] Progressive resistive training has been shown to improve lean mass and biochemical parameters in patients with high levels of inflammation. Whether any of these therapies could improve outcomes in the clinically advanced PAD population is currently unknown.

CONCLUSIONS

The risk model we have presented containing parameters of inflammation, nutrition, and renal function has excellent discriminatory ability for midterm all-cause mortality in patients selected to undergo lower extremity arterial bypass surgery. Among all markers of inflammation, hsCRP has proven the most robust and is most representative of the integrated inflammatory response. A significant minority of patients undergoing lower extremity bypass surgery present with a hypercytokinemic, hypoalbuminemic vascular cachexia that places them at a particularly increased risk for death. Whether there are any therapeutic mitigating strategies to decrease the inflammatory response or improve the biochemical parameters in these high-risk patients remains to be tested.

AUTHOR CONTRIBUTIONS

Conception and design: CO, MB, MC, MSC
Analysis and interpretation: CO, JK, NH, WG, MB, MC, MSC
Data collection: CO, JK, WG, MSC
Writing the article: CO, JK, WG, MSC
Critical revision of the article: CO, MSC, NH
Final approval of the article: CO, MSC
Statistical analysis: CO, NH, MSC, WG
Obtained funding: MC, CO, MSC
Overall responsibility: CO

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