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Low Levels of A Natural IgM Antibody are Associated with Vein Graft Stenosis and Failure

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Abstract

Introduction—All humans have natural, protective antibodies directed against phosphorylcholine (PC) epitopes, a common inflammatory danger signal appearing at sites of cell injury, oxidative stress, and on bacterial capsules. In large human cohorts, low levels of anti-PC IgM were associated with a significantly increased risk of stroke or myocardial infarction. However it is not known if these antibodies protect against the premature closure of arterial reconstructions.

Methods—A prospective, observational study of patients undergoing elective, infrainguinal, autogenous vein bypasses for atherosclerotic occlusive disease of the legs was conducted. Clinical data were recorded prospectively, and preoperative levels of anti-PC IgM measured with the CVDefine kit from Athera Biotechnologies. The principal clinical endpoint was the loss of primary patency (loss of graft flow, or any intervention for stenosis). Patients were followed regularly by duplex ultrasound at 1, 3, 6, 12, 18 months, and yearly thereafter.

Results—Fifty-six patients were studied, for an average of 1.3 years. Indications for surgery were claudication (33.9%), ischemic rest pain (17.9%), and ischemia with ulceration or gangrene (48.2%). Seventeen (30.4%) patients experienced loss of primary patency (10 graft occlusions, 7 surgical or endovascular revisions of graft stenoses). Kaplan-Meier survival analysis showed that the quartile of patients with the lowest anti-PC IgM levels had significantly worse primary graft patency (p=.0085, log rank test). Uni- and multivariate Cox proportional hazards analysis revealed that the preoperative anti-PC IgM level was an important predictor of graft failure. Patients with IgM values in the lowest quartile had a 3.6-fold increased risk of graft failure (95% confidence interval: 1.1-12.1), even after accounting for other significant clinical or technical factors such as indication for surgery, site of distal anastomosis, or vein graft diameter.

Conclusions—A naturally occurring IgM antibody directed against the pro-inflammatory epitope phosphorylcholine may be protective against vein graft stenosis and failure, through anti-

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inflammatory mechanisms. Measurement of this antibody may be a useful prognostic indicator, although larger studies of more diverse populations will be needed to confirm these results. The biological actions of anti-PC IgM suggest it may be useful in developing immunotherapies to improve bypass longevity.

Introduction

Atherosclerosis is an inflammatory, immune-mediated disease[1-3]. The appearance of oxidized lipids and related pathologic neo-antigens in the vessel wall are commonly the inciting events that attract inflammatory cells to the site of injury, beginning a cascade of inflammatory events that lead to intimal thickening, smooth muscle cell proliferation, matrix deposition and ultimately complex plaque formation[3, 4]. Our increasing knowledge of arterial injury and repair has led to an understanding that there are common mechanisms of cellular and humorally-mediated inflammation that govern primary atherosclerosis, neointimal hyperplasia, and adaptive vascular remodeling. While much research has emphasized the pro-inflammatory mediators, some natural anti-inflammatory mechanisms have also been known for some time[5].

Among these are natural IgM antibodies that belong to the innate immune system, and are present at birth[6-8]. These natural antibodies are programmed to recognize a restricted pattern of “danger signals”, and hence protect against bacterial invasion, control oxidative stress, and neutralize pro-inflammatory oxidized lipid moieties[9, 10]. As outlined by Lutz and others[8], during oxidative stress at sites of vascular injury, the abundant lipid phosphatidylcholine undergoes oxidation to expose normally hidden phosphorylcholine (PC) headgroups. Dying cells and cellular debris also expose and release this neo-antigen of phosphorylcholine, which is recognized by the immune system as “altered self”, and instigates a complex inflammatory response[5]. These normally cryptic PC epitopes are also a prominent part of the capsular polysaccharide of many bacteria. Thus, abnormally exposed PC is one of the major antigens recognized by our innate immune system[11]. Research and diagnostic tools have now been developed to study natural IgM that recognizes exposed phosphorylcholine (PC) in humans and animals[12]

The putative vascular protective mechanisms of this antibody include the inhibition of macrophage uptake of oxidized LDL, blockade of macrophage IL-6 production[13], and suppression of endothelial activation by inflammatory mediators[10, 14]. *In vivo*, anti-PC IgM is thought to attenuate the inflammatory response to PC that is exposed at sites of vascular injury. Low levels of anti-PC IgM may thus lead to excessive activation of inflammatory responses. In large human cohorts, low levels of anti-PC IgM were associated with a significantly increased risk of stroke or myocardial infarction[14-16, 16, 17], and mortality in dialysis patients[18]. Furthermore, in animal models, enhancement of anti-PC antibodies through immunization or supplementation reduced atherosclerosis and intimal hyperplasia in a mouse vein bypass model[19, 20]. Twin studies suggest that forty percent of the variability in human anti-PC IgM levels is attributable to genetic variance[21].

Autogenous vein grafts used to bypass atherosclerotic occlusive disease of the lower extremities are prone to early failure due to stenosis and thrombosis. Atherosclerosis, *per se*, is not the cause of these early failures, which typically occur within the first one to two years after surgery[22]. However both early graft failure and atherosclerosis have in common the derangement of the injury/repair process by activation of thrombo-inflammatory pathways, leading to intimal hyperplasia, smooth muscle cell proliferation, and induction of a prothrombotic local vascular environment. Thus, it is possible that anti-PC IgM antibodies might modulate and normalize the vascular healing process in vein grafts, just as they modulate the progression of cardiovascular arterial disease. Previous human studies

illustrated the importance of low levels of anti-PC IgM, as they were associated with loss of atheroprotection, and adverse events. We conducted this pilot study to determine if there was a relationship between low levels of anti-PC IgM antibodies, and the loss of primary patency of infrainguinal autogenous vein grafts.

Patients and Methods

We designed a prospective, longitudinal observational study of all eligible patients undergoing infrainguinal bypass at a single institution. After informed consent, patients scheduled for elective, infrainguinal bypass surgery using autologous vein were prospectively recruited at the VA Puget Sound Health Care System, a large regional referral center for vascular surgery. The research was approved by the Institutional Review Board of the Human Research Protection Program of the VA Puget Sound. All elective infrainguinal bypasses using autogenous vein were eligible for study. Exclusion criteria for the study were: operations for non-atherosclerotic or aneurysmal disease, concurrent aorto-bifemoral or femoral-femoral bypass, use of a prosthetic bypass conduit, active treatment for malignancy (i.e. chemotherapy), chronic hemodialysis, perioperative thrombosis of the graft, or a diagnosed systemic inflammatory disease (e.g. lupus or other treatment with immunosuppressive drugs) or thrombophilia. At surgery, completion intraoperative imaging was performed in all cases (angiography or duplex ultrasound) to confirm patency and freedom from stenosis or technical defects. Patients were classified as diabetic if the diagnosis was in their problem list and they were being chronically treated with an oral hypoglycemic agent or insulin. Active smoking was self-reported by the patient. We included as “smokers” those who had quit in the past week prior to surgery. The non-smoker classification included those former smokers who had quit months to years previously.

The clinical severity of their occlusive disease at the time of surgery (Leg Status) was classified as *claudication* (Fontaine stage II, Rutherford grade I), *rest pain* (Fontaine stage III, Rutherford grade II), or critical ischemia with *ulceration* (Fontaine stage IV, Rutherford grade III), according to the guidelines of the Society for Vascular Surgery[23]. Several other surgical factors known to influence the primary patency of lower extremity bypass grafts were also recorded prospectively: a history of a previously failed bypass graft, the location of the distal anastomosis, and the minimum diameter of the pressurized vein conduit. Finally, technical aspects of the surgical procedures that have been associated with a higher risk of failure were noted, which we denoted as Higher Risk Vein Graft. This factor was considered positive if veins other than the greater saphenous vein were used in part or whole *or* if there were any technical problems during surgery requiring intraoperative revisions.

The long term patency of the grafts was monitored by arterial color duplex ultrasound and ankle/brachial indices at 4-6 weeks, then at 3, 6, 12, 18 months, and yearly thereafter. Standard duplex graft surveillance was performed on Acuson Antares equipment, using protocols established by Bandyk *et al.*[24, 25]. The primary clinical endpoint was the loss of primary patency (graft failure), defined as any graft revision to maintain patency, or graft thrombosis. The first of these events to occur triggered the primary endpoint. Graft revision was defined as any open surgical or endovascular procedure performed on a patent graft to correct stenosis or narrowing. The treating surgeon made the independent clinical decision regarding the need for revision, based on the accepted definitions for a hemodynamically significant vein graft stenosis (i.e. diameter reduction of >75%, peak systolic velocity > 300 cm/sec, velocity ratio > 3.5)[25]. Graft occlusion was defined (per published guidelines[23]) as clinical loss of flow, which was confirmed by complementary imaging when necessary.

Peripheral blood samples were obtained to measure anti-PC IgM preoperatively, using evacuated tubes containing potassium-EDTA (1.8mg/ml final concentration, Becton

Dickinson). Within one hour of collection, the tubes were centrifuged at 200×g for 10 minutes at room temperature, and the platelet-rich plasma transferred to polypropylene microcentrifuge tubes. These tubes were centrifuged at 13,000×g for 10 minutes, and the platelet-free plasma was removed, aliquoted, and stored at -70°C until assay. The manufacturer of the kits states that samples tolerate at least 3-4 cycles of freeze thawing, although this was avoided by making aliquots. IgM anti-PC is also stable for years at -70 °C. Anti-PC IgM levels were determined by ELISA using the CVDefine kit (Athera Biotechnologies)[12], an enzyme-linked immunosorbent assay which employs an immobilized, conjugated phosphorylcholine antigen, and human anti-PC IgM for standards. Briefly, thawed plasma was centrifuged 5 minutes at 13,000×g to remove aggregates, then diluted as directed in the provided assay diluent. Samples, standards, and controls provided in the kit were added in duplicate to microwells coated with PC. After washing, the bound IgM was reacted with anti-IgM conjugated to horseradish peroxidase, measured with TMB as substrate. The standard curve was generated using the cubic spline algorithm (GraphPad Prism), and sample concentrations were expressed as arbitrary units/ml as defined by the manufacturer.

Statistical Analyses

Analyses were conducted in the statistical software package R (version 2.15.2). Survival analyses were made with the Kaplan–Meier survival curve and the Cox proportional hazard model, stratified by dichotomization of anti-PC IgM values into the lowest 25% and the remaining 75%. This was justified by the biological behavior of this IgM antibody, as observed in clinical studies. The univariate and multivariate Cox regression analyses are presented as hazard ratios [HR; 95% confidence intervals (CI)]. Cox adjustments were done with correction for the known predictors of graft failure outlined below. Values of IgM were log-transformed where noted. Log rank tests were also completed to determine whether the survival distributions between groups were equal. To determine if the value of pre-operative IgM was associated with clinical characteristics of the patients, uni- and multivariate linear regression models were completed for each clinical parameter.

Results

Patient Characteristics

A total of 68 patients were initially recruited to the study. Eight of these patients were excluded from further study because they did not have a qualifying operation (e.g. prosthetic graft, or never had surgery), 3 had perioperative occlusions before discharge, and 1 was excluded for lack of intraoperative completion imaging. All of the subsequent primary endpoints in the remaining 56 patients occurred more than 30 days after their surgery. Thus, 56 consecutive, eligible patients were enrolled in the study between 2007 and 2012, and all had their levels of plasma anti-PC IgM antibodies measured preoperatively. Fifty-four subjects were Caucasian, two African-American, and three were female. Table I describes the main characteristics of these subject's medical conditions, their mean and median follow up, and the relevant details of their surgeries. Thirteen patients received the designation of Higher Risk Vein Graft, based on technical aspects of the surgery (veins other than the greater saphenous, 3 patients) *or* any technical problems requiring intraoperative revisions (10 patients). Technical revisions included autogenous patch angioplasties of narrow anastomoses, and intraoperative repairs or resections of venous conduit abnormalities.

Relationship between Anti-PC IgM Levels and Outcomes

The average preoperative IgM value was 74.4 units/ml (± 11.9 sem) and the median was 38.0 units/ml. The values were not normally distributed, so a log transformation was

completed and the log values were normally distributed. Seventeen of the fifty-six patients experienced a primary endpoint (30.4%), of which ten were graft occlusion, and seven graft revisions for a hemodynamically significant stenosis. The clinical characteristics of these patients are described in the on-line, Supplemental Table I.

The primary patency of the overall group is presented as a Kaplan-Meier survival curve in Figure 1. For survival analysis of the effect of anti-PC IgM, the preoperative valueswere dichotomized, comparing those in the lowest quartile (n=14) to the other 75% (n=42). This analytic approach was taken because prior research suggests that low levels of IgM are specifically associated with the loss of protective effects, while a much wider range of higher levels are associated with freedom from adverse outcomes. Those patients in the lowest quartile experienced a significantly inferior primary graft survival, compared to the higher 75% (p=.0085, log rank test, Figure 2). We tested alternative dichotomization schemes, stratifying patients by the lowest quintile versus the highest 80%, or those below the median value versus those above. Each of these analyses (log rank test 0.019 and 0.035 respectively) were statistically significant, but stratification by the lowest quartile gave the best discrimination of clinical outcome. Graft survival was also analyzed from the perspective of freedom from intervention for stenosis, excluding those primary endpoints that arose due to graft thrombosis. Figure 3 illustrates the Kaplan-Meier curves, and shows that those patients in the lowest quartile of anti-PC IgM values had a higher incidence of graft stenosis (log rank test 0.023) .

The preoperative IgM values were significantly lower in those subjects who suffered loss of primary patency (median 27.9 units/ml, 20.45-55.08 interquartile range [IQR]) compared with those whose grafts remained patent (median 46.1, 34.78-134.02 IQR). Because the majority of graft failures occur within the first year of follow up, we included only the 42 subjects with at least one full year of surveillance of their patent grafts. Figure 4 illustrates these differences, which were significant (P=.015, Mann-Whitney rank sum test), and also illustrates a scatter plot of all preoperative values.

To identify the specific factors that might be associated with the duration of primary patency, we performed Cox proportional hazards analyses of the entire cohort of 56 patients, first testing individually the variables that might affect the loss of primary patency (Table II). The variables that were individually significant at alpha level .05 were distal anastomosis (P=.02, tibial vs. above knee), minimum vein diameter (P=.001), and pre-operative IgM. The variables of Leg Status (P=.06, ulceration vs. claudication) and high risk vein graft (P=.07) were of borderline significance, so they were included in the multivariate Cox proportional hazards model. Subsequent multivariate analysis yielded a parsimonious model for graft failure in which the minimum vein graft diameter, and the preoperative anti-PC IgM value were the two most significant factors associated with graft failure. Table III shows that patients in the lowest quartile of anti-PC IgM values (below ~27 units/ml) had a 3.62 fold greater risk of graft failure than the other 75%, even accounting for the diameter of vein conduit, leg status, site of distal anastomosis, and high risk vein graft characteristics. Also, for each 1 mm reduction in vein graft diameter from the median (4 mm), the risk of graft failure increases by 5.56 fold, after adjustment for distal anastomosis, high risk vein graft characteristics and preoperative IgM quartile.

We conducted a similar analysis, considering exclusively the endpoint of graft stenosis greater than 75% - all of which underwent intervention and lost primary patency. Tables IV and V illustrate the univariate and multivariate analyses. By univariate analysis, the preoperative IgM quartile was significant, and the characteristics of Prior Failed bypass and High Risk Vein Graft were of borderline significance. Including all these in a multivariate

analysis shows that those patients in the lowest quartile of IgM values had a 7.8 fold increased risk of graft stenosis requiring revision.

Relationship Between Clinical Factors and IgM Levels

Patients with different clinical characteristics (e.g. diabetes, or indication for surgery) might disproportionately have higher or lower IgM values. To investigate these relationships, we performed univariate and multivariate linear regressions. Neither analysis revealed any significant statistical relationship between IgM values and the clinical characteristics (age, Leg Status, Diabetes, Smoking, Distal Anastomosis, Prior Failed Graft, High Risk Vein Graft, and Minimum Diameter). These results are summarized in the on-line Supplemental Table II. The values of preoperative anti-PC IgM were not significantly different between diabetics/non-diabetics, or according to indication for surgery (on-line Supplemental Table III). Twenty-eight percent of the lowest quartile of IgM values had high risk bypasses, and 21% of the highest three quartiles had high risk bypasses. This difference was not significant (Fisher's exact test, p=1.0).

Discussion

This most durable of infrainguinal revascularization procedures, lower extremity bypass with vein, is still prone to stenosis and graft failure that can affect 20-35% of infrainguinal grafts within the first year of surgery[22, 26-28]. In this prospective, longitudinal study, we have shown that the preoperative level of a naturally occurring IgM antibody directed against the inflammatory antigen, phosphorylcholine, is closely associated with the primary patency of autogenous infrainguinal vein grafts. Even accounting for other well-known factors that influence the longevity of bypass grafts, such as the severity of occlusive disease, the quality of the vein conduit, and technical aspects of the surgery, the preoperative anti-PC IgM value was a significant predictor of loss of primary patency. When the analysis was restricted to loss of primary patency due to hemodynamically significant vein graft stenosis, the results were similar.

We confirmed that Fontaine class, the site of the distal anastomosis, vein diameter and intraoperative technical factors, when considered individually, may be prognostic indicators of future vein graft stenosis and occlusion[29-32]. They are useful in postoperative decision making regarding antithrombotic therapy and the intensity of graft surveillance. Until now, biological measurements that directly reflect a patient's predisposition to graft stenosis or occlusion have been lacking. Our data suggest that the preoperative level of anti-PC IgM may be another potential tool for prognostication and postoperative management. The mechanisms of action of this natural IgM may also lend new insights into the mechanisms of graft failure, and the development of preventative strategies.

Previous studies of anti-PC IgM and cardiovascular disease have shown that low levels are most predictive of adverse outcomes. Many studies suggest that a wide range of IgM levels might be healthy, while levels below a certain threshold lead to the loss of anti-inflammatory, atheroprotective effects, which is why we analyzed the IgM values as a discrete variable. Fiskesund and colleagues, in a study of 227 subjects with 455 matched controls found the strongest association between IgM and first stroke below the 30th percentile of IgM values. The association with stroke was more pronounced at lower percentiles and primarily in women but not in men[15]. Population-based studies involving almost 5,000 subjects, from the Malmo Diet and Cancer Study cohort and a Stockholm screening project, found that low levels of anti-PC IgM were most strongly associated with cardiovascular events in men[14, 33]. The interactions between gender and anti-PC IgM effects are still unclear. Women in general have higher levels of this IgM, which may be one factor

contributing to the lower incidence and later onset of vascular disease in females[33, 34]. Our current study cannot resolve this issue, as our subjects were predominantly male.

The vascular-protective mechanisms of anti-PC IgM center on its ability to block cellular inflammatory responses to phosphorylcholine. This epitope is normally hidden, but becomes exposed when low density lipoprotein becomes oxidized, when there is cellular damage and death, or when certain bacteria are present. Su *et al.* found that the expression of adhesion molecules by human endothelial cells in response to platelet activating factor (itself a PC derivative) was inhibited by anti-PC IgM[35]. de Faire and colleagues demonstrated that anti-PC IgM blocked the uptake of oxidized LDL by macrophages[14]. Research in animal models has shown that inducing higher levels of anti-PC IgM retards the progression of atherosclerosis, and prevents vein graft thickening, providing evidence of a cause and effect relationship[20, 36]. These studies suggest that the innate anti-PC IgM antibody may play an important role in the modulation of vascular inflammation.

As in atherosclerosis, vascular inflammation is a key factor in graft failure. Although the etiologies of autogenous graft failure are complex and diverse, loss of primary patency in the first two years is primarily the result of neointimal hyperplasia and constrictive remodeling[37, 38]. The activation of inflammatory and thrombotic pathways (which are tightly intertwined) is thought to be a prime driver of the derangements of vascular healing that result in graft stenosis and failure[22, 39]. Patients with peripheral arterial occlusive disease (PAD) are known to have pathological activation of these thrombo-inflammatory pathways[40, 41].

Synthesizing our current knowledge – that anti-PC IgM plays an anti-inflammatory role against the appearance of a common inflammatory molecule; that vascular inflammation contributes to graft stenosis and failure; and that PAD patients have exaggerated inflammatory profiles – the results of our current study are consistent and confirmatory. Patients with the lowest levels of anti-PC IgM were significantly more prone to graft failure than those with higher levels.

Biological strategies to abrogate the deranged response to injury have uniformly failed in humans, and the human variability in the response to vascular injury is poorly understood. The biological actions of anti-PC IgM raise the possibility of new strategies to normalize vascular healing, by suppressing of one of the earliest events in vascular healing – the exposure of inflammatory phosphorylcholine. Such strategies could include conferring passive immunity by the administration of an engineered anti-PC antibody to those patients with low levels, or the induction of enhanced immunity by vaccination. Both strategies have been successful in animal models[19,20].

This study has limitations. This was an observational study, so we did not dictate patient management or antithrombotic treatments. Some variables, like the use of aspirin (92.9%) and statins (80.4%) were so highly prevalent that analysis of their influence on primary patency was not possible. The study population does not shed light on possible gender or racial differences in anti-PC IgM levels, nor the prognostic significance of such factors. We are currently conducting larger and broader studies to address these questions. Despite these limitations, and in spite of a relatively small cohort, the relationship between anti-PC IgM levels and loss of primary patency was strong and consistent, even after accounting for other important variables.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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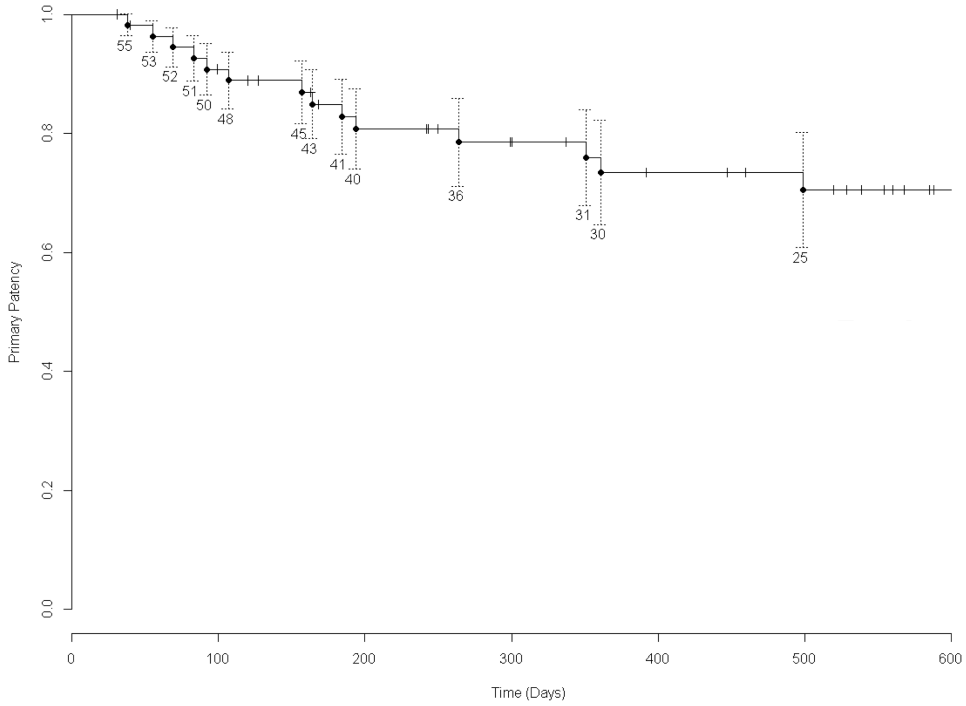

Figure 1. Kaplan-Meier Survival Analysis of Overall Primary Graft Patency

Freedom from loss of primary patency is illustrated for all 56 patients studied. The numbers above and below the graph lines indicate the number of subjects at risk at each interval. SEM's are shown.

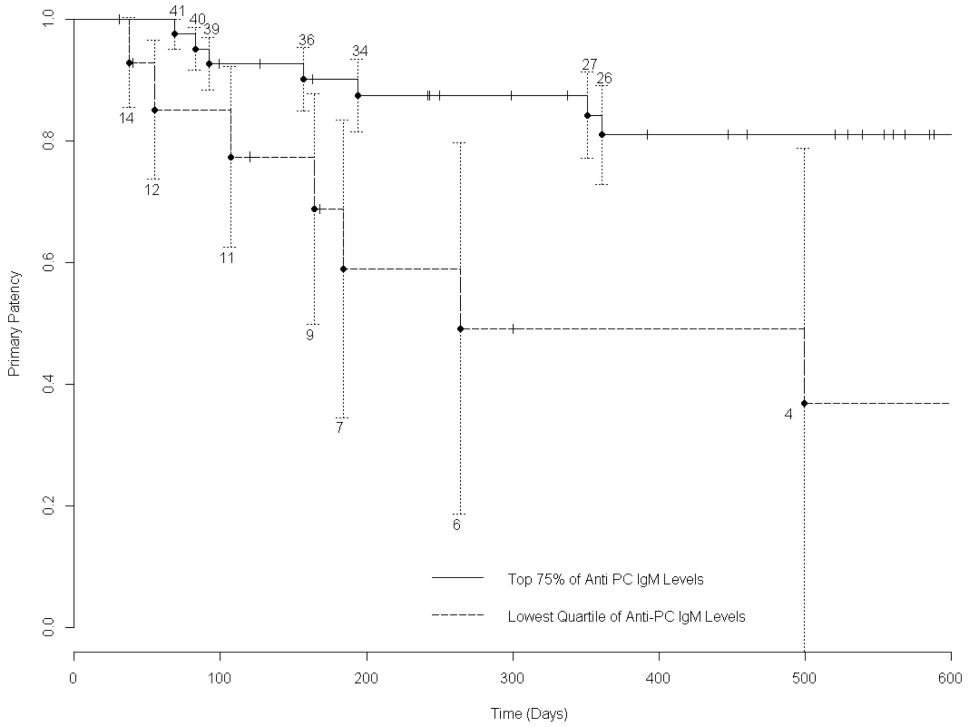

Figure 2. Kaplan-Meier Survival Analysis of Overall Primary Graft Patency According to IgM Level

The difference in primary graft patency (stenosis requiring intervention, and graft occlusion) between those in the bottom quartile of anti-PC IgM values, and those in the top 75% was significant (p=.0085 log-rank test). The numbers above and below the graph lines indicate the number of subjects at risk at each interval. SEM's are shown, with truncation of the graph to 600 days, where the SEM of the primary group exceeds 10%.

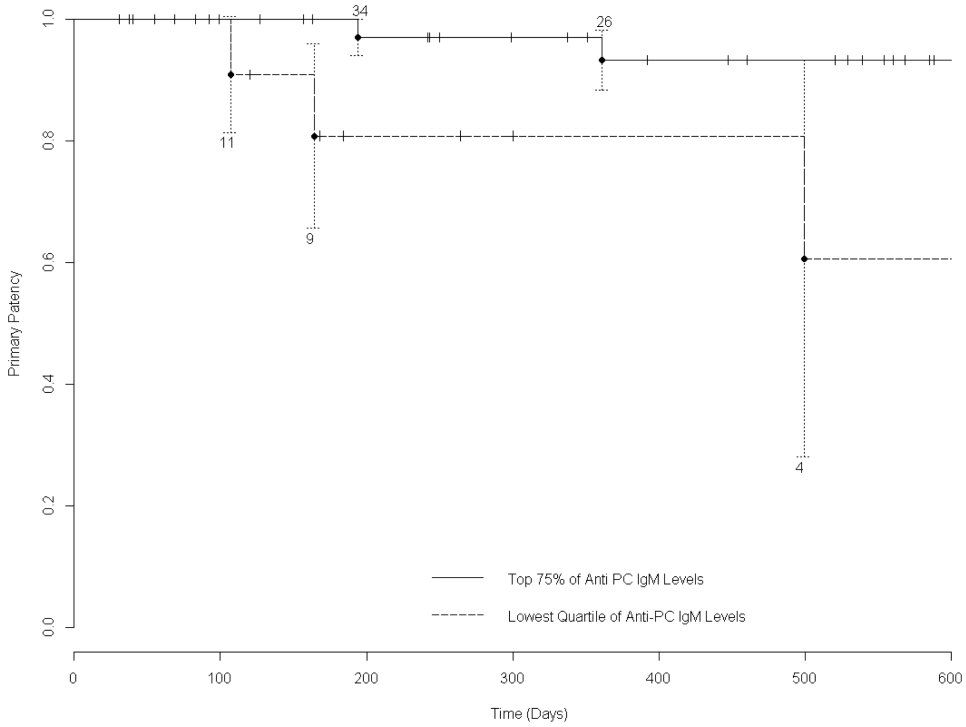

Figure 3. Kaplan-Meier Survival Analysis of Freedom from Stenosis According to IgM Level
Considering the endpoint of stenosis (requiring intervention) alone, the differences in graft survival were significant (p=.023 log-rank test). Technical details of the graph are the same as figure 2.

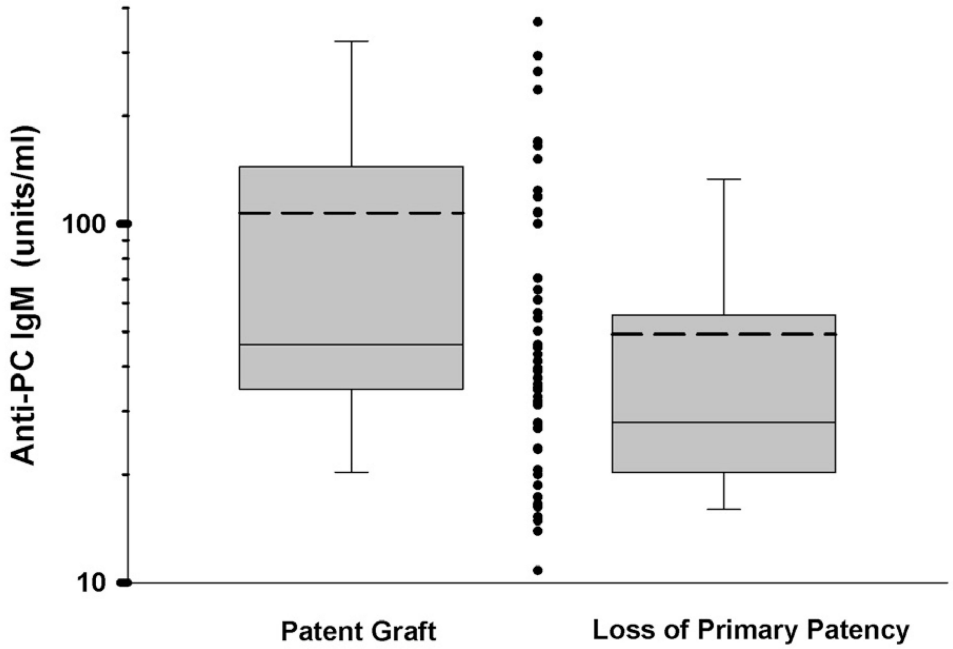

Figure 4. Anti-PC IgM Levels According to Primary Graft Patency

42 subjects with at least one full year of follow-up were included. The box plots show the mean (dotted line), median (solid line), 25-75 percentile (box), and 10-90 percentile (error bars). P= .015, Mann-Whitney rank sum. The central scatter plot illustrates the range of preoperative IgM values among all 56 patients.

Table I
Clinical and Surgical Characteristics of Patient Population

Characteristic	Mean	Median	Range or Percentage
Age	64.8	63.0	50 – 81
Follow Up - All Patients (days)	464	377	31 – 1580
Time to Loss of Primary Patency (days)	305	184	38 – 1261
Clinical Factors			
Smoking			55.4%
Diabetes			37.5%
Hypertension			87.5%
Hyperlipidemia			87.5%
Statin use			80.4%
Aspirin			92.9%
Clopidogrel			8.9%
Warfarin			7.1%
Leg Status			
Claudication			33.9%
Rest Pain			17.9%
Ulceration			48.2%
Ankle-Brachial Index	0.46	0.47	0-0.96
White Blood Cell Count (K/uL)	8.2	7.7	5-13.6
Prior Failed Graft			12.5%
Operative Factors			
Above Knee distal anastomosis			33.9%
Below Knee distal anastomosis			28.6%
Tibial distal anastomosis			37.5%
High risk vein graft			23.2%
Diam. Of Vein (mm)	4.06	4.00	3-7

Table II

Univariate Cox Proportional Hazards Model for Primary Endpoint – Loss of Primary Patency

These clinical characteristics were tested individually for their relationship to the occurrence of the primary endpoint over time. The preoperative IgM value, whether considered as the lowest quintile, quartile, or below the median, was significantly associated with loss of primary patency. The following factors were chosen for inclusion in the subsequent multivariate hazards model because they were significant, or approached significance: Leg status, Distal anastomosis, High risk vein graft, Minimum diameter, and lowest quartile of IgM value.

Variable	Level	Hazard Ratio	Lower 95%	Upper 95%	P-value for Z statistic
Age	-	1.04	0.98	1.11	0.22
Leg Status (reference level = claudication)	Rest Pain	1.81	0.40	8.13	0.44
	Ulceration	3.20	0.96	10.67	0.06
Diabetes	Yes	1.54	0.59	3.99	0.38
Smoker	Yes	1.03	0.39	2.71	0.96
Distal Anastomosis (reference level = above knee)	Below knee	1.94	0.43	8.73	0.39
	Tibial	5.05	1.29	19.77	0.02
Prior Failed Graft	Yes	2.22	0.70	7.00	0.18
High Risk Vein Graft	Yes	2.53	0.91	7.02	0.07
Index Leg Ankle Brachial Index (for each .10 decrease)	-	1.19	0.96	1.48	0.11
Minimum Diameter	-	0.23	0.10	0.56	0.001
Pre-operative IgM value – Quintile	Lowest 20%	3.37	1.15	9.87	0.03
Pre-operative IgM value – Quartile	Lowest 25%	3.53	1.30	9.58	0.01
Pre-operative IgM value – Median	Below Median	2.94	1.03	8.40	0.04

Table III

Multivariate Cox Proportional Hazards Model for Primary Endpoint – Loss of Primary Patency

In this multivariate hazards model, Minimum diameter of the vein graft, and the lowest quartile of preoperative IgM values were significantly associated with loss of primary patency.

Variable	Level	Hazard Ratio	Lower 95%	Upper 95%	P-value for Z statistic
Leg Status (reference level = claudication)	Rest Pain	1.07	0.21	5.40	0.93
	Ulceration	1.40	0.36	5.48	0.63
Distal Anastomosis (reference level = above knee)	Below knee	2.68	0.56	12.74	0.22
	Tibial	2.86	0.59	13.87	0.19
High Risk Vein Graft	Yes	1.51	0.45	5.09	0.50
Minimum Diameter (for each decrement of 1mm from median)	-	5.56	1.94	15.95	0.001
Pre-operative IgM value – quartile	Lowest 25%	3.62	1.08	12.07	0.037

Table IV

Univariate Cox Proportional Hazards Model for Incidence of Graft Stenosis

These clinical characteristics were tested individually for their relationship to the occurrence of the endpoint of graft stenosis. The following factors were chosen for inclusion in the subsequent multivariate hazards model because they were significant, or approached significance: Prior failed graft, High risk vein graft, and lowest quartile of IgM value.

Variable	Level	Hazard Ratio	Lower 95%	Upper 95%	P-value for Z statistic
Age	-	1.04	0.94	1.15	0.49
Leg Status (reference level = claudication)	Rest Pain	0.82	0.085	7.96	0.87
	Ulceration	1.74	0.32	9.61	0.53
Diabetes	Yes	0.69	0.13	3.57	0.66
Smoker	Yes	1.63	0.31	8.44	0.56
Distal Anastomosis(reference level = above knee)	Below knee	1.01	0.17	6.06	0.99
	Tibial	1.33	0.2	8.98	0.77
Prior Failed	Yes	3.87	0.65	23.23	0.14
High Risk Vein Graft	Yes	4.92	0.96	25.09	0.06
Minimum Diameter (for each decrement of 1mm from median)	-	2.07	0.56	7.62	0.27
Index Leg Ankle Brachial Index (for each .10 decrease)	-	1.07	0.74	1.54	0.73
Pre-operative IgM value – Quartile	Low 25%	5.47	1.07	27.87	0.04

Table V

Multivariate Cox Proportional Hazards Model for Incidence of Graft Stenosis

In this multivariate hazards model, the lowest quartile of preoperative IgM values was significantly associated with graft stenosis requiring intervention.

Variable	Level	Hazard Ratio	Lower 95%	Upper 95%	P-value for Z statistic
Prior Failed	Yes	3.45	0.39	30.83	0.27
High Risk Vein Graft	Yes	3.5	0.47	26.03	0.22
Pre-operative IgM value – quartile	Low 25%	7.79	1.27	47.82	0.027
					Log rank test, P = 0.013 for this model