

Anti-phosphorylcholine IgM, an Anti-inflammatory Mediator, Predicts Peripheral Vein Graft Failure: A Prospective Observational Study[☆]

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WHAT THIS PAPER ADDS

In a longitudinal, prospective, observational study of 142 patients undergoing leg bypass with autogenous vein, it was found that patients with the lowest pre-operative levels of an innate IgM antibody directed against phosphorylcholine (anti-PC) were twice as likely to suffer loss of primary patency, after controlling for other risk factors of graft failure. This naturally occurring biological mediator may be a useful marker to identify patients at higher risk of graft failure, and offers the potential for novel, directed therapies for vascular inflammation and its consequences.

Objectives: One third of infrainguinal vein bypasses may fail within the first 1.5 years. Pro- and anti-inflammatory mechanisms are thought to be involved in these graft stenoses and occlusions. In previous studies, low levels of anti-phosphorylcholine IgM (anti-PC IgM, an innate anti-inflammatory IgM) have been associated with increased cardiovascular events. In this study, the peri-operative dynamics of anti-PC IgM levels were established during leg bypass surgery, and associations assessed between anti-PC IgM levels and primary graft patency.

Design and methods: This was a prospective, observational cohort study of infrainguinal autogenous vein bypass for peripheral arterial occlusive disease involving four university affiliated hospitals. Plasma cytokine and anti-PC IgM levels were measured pre- and post-operatively. The outcome of interest was loss of primary graft patency because of occlusion or intervention for graft stenosis.

Results: One hundred and forty-two consecutive patients were enrolled: mean age 66 (46–91); 91% white race and male; 72.5% critical limb ischaemia (Fontaine III or IV). Median pre-operative anti-PC IgM levels were 49 units/mL (IQR 32.3–107.7, mean 89.8 + 101 sd). During follow up of an average of 1.8 years (1 month–7.4 years), 50 (35.2%) grafts lost primary patency. Pre-operative levels of interleukin 6 or C-reactive protein did not predict graft failure. Patients with pre-operative anti-PC IgM values in the lowest quartile had a twofold increased risk of graft failure (multivariable Cox proportional hazard, $p = .03$, HR 2.11, 95% CI 1.09–4.07), even after accounting for the other significant factors of conduit diameter, distal anastomosis, smoking, and the severity of leg ischaemia.

Conclusions: Low levels of anti-PC IgM are associated with vein bypass graft failure. This biological mediator may be a useful marker to identify patients at higher risk, and offers the potential for novel, directed therapies for vascular inflammation and its consequences.

Keywords: Anti-inflammatory agents, Graft occlusion, Vascular, Inflammation, Saphenous vein, Vascular grafting, Vascular patency

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INTRODUCTION

Pathological inflammation within the arterial wall has long been implicated in chronic peripheral arterial disease (PAD),

as well as in the failure of endovascular interventions and vein grafts.¹ Although statins are known to have pleiotropic anti-inflammatory effects,² much less is known about innate anti-inflammatory mechanisms that can attenuate these pathological responses to vessel wall injury. The human innate immune system includes a family of antibodies that naturally recognise danger associated molecular patterns (DAMPs), which are localised inflammatory signals liberated by oxidised phospholipids, and by cell injury and apoptosis.^{3,4} Among these DAMPs is the polar headgroup (phosphorylcholine or PC) of the membrane phospholipid

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phosphatidylcholine. PC is a common inflammatory component of damaged cell membranes.⁵ This normally hidden phospholipid is exposed during cell injury and death, and serves to instigate a cascade of local, inflammatory cellular events.⁶ As a component of the innate immune system, humans have a naturally occurring IgM antibody that specifically targets PC.⁷ This antibody is believed to play an anti-inflammatory role, by attenuating the inflammatory response to the danger signal, and facilitating clean up and repair at sites of cell injury. Low levels of this antibody have been associated with an increased risk of myocardial infarction and stroke in large population based studies.^{8–10} Likewise, studies suggest that higher levels of this antibody may attenuate the inflammatory response to injury: these antibodies reduce macrophage activation *in vitro*,^{8,11} and reduce intimal hyperplasia in animal models.¹²

To date, these innate, anti-inflammatory antibodies have been studied mainly in the context of chronic atherosclerosis. Little is known about their role in the vascular response to injury, for example in peripheral arterial vein grafts. In a previous pilot study,¹³ a positive relationship was found between low levels of this anti-PC IgM and peripheral vein graft failure. To expand on this initial finding, a larger, longitudinal, prospective, observational cohort study of patients undergoing lower extremity vein graft bypass for arterial occlusive disease has now been completed. The goals of this study were to establish the dynamics of anti-PC IgM levels throughout the peri-operative period, and to determine whether the plasma levels of this anti-inflammatory IgM antibody correlate with long-term graft patency, freedom from stenosis and occlusion.

MATERIALS AND METHODS

A prospective observational cohort study

This was a prospective, observational, longitudinal cohort study of patients undergoing infrainguinal bypass with autogenous vein. All subjects were recruited from the VA Puget Sound Health Care System and the University of Washington hospitals. Recruitment was between 2008 and 2016, and the study end date was December 31, 2017. The procedures and protocols were approved by the Institutional Review Boards for human studies at the University of Washington, and the VA Puget Sound Health Care System. All subjects gave their informed consent.

Eligibility

All consecutive subjects scheduled for elective infrainguinal bypass with autogenous vein for symptomatic peripheral arterial occlusive disease were eligible, were identified from surgical schedules, and recruited before surgery.

Exclusions

Subjects were not recruited if they were on haemodialysis, had a confirmed diagnosis of any autoimmune disease (e.g. rheumatoid arthritis or lupus), or any diagnosed prothrombotic diathesis, or if the operation was for peripheral

aneurysmal disease. Subjects were excluded post-operatively if the planned autogenous bypass was not performed (i.e. prosthetic graft), if the bypass was combined with open surgical bypass of the iliac arteries or aorta, or if no satisfactory intra-operative completion imaging was performed. Grafts that occluded within the first 30 days after surgery were also excluded, because these failures are primarily technical in nature.¹⁴

Follow up

Patients were followed regularly at clinically prescribed intervals to assess for graft patency. Standardised duplex ultrasound surveillance examinations of the graft were performed at 1, 3, 6, 12, and 18 months, then yearly thereafter, according to the procedures and principles outlined by Bandyk.¹⁵ Their published guidelines for critical graft stenoses were followed (peak systolic velocity > 300 cm/s, velocity ratio > 3.5), but the final decision for graft intervention was left to the judgement of the treating surgeon. The treating clinicians were blinded to the results of the IgM and cytokine measurements. Follow up indices were calculated for all subjects according to von Allmen et al.¹⁶ The IgM quartile was not associated with cohort attrition by regression analysis, so losses to follow up were censored in Cox proportional hazards analysis.

Endpoints, covariables

The outcome of interest was loss of primary patency. This was defined as “graft failure” (endpoints were either thrombosis, or a surgical or endovascular intervention for a critical vein graft stenosis). After primary graft failure, follow up was ended, and secondary or assisted patency was not recorded.

The clinical indications (i.e. original rationale) for surgery were classified according to the guidelines of the European Society for Vascular Surgery:¹⁷ claudication (Fontaine stage II, Rutherford Grade I), ischaemic rest pain (Fontaine stage III, Rutherford Grade II), or critical limb ischaemia with tissue loss (Fontaine stage IV, Rutherford Grade III). The severity of leg ischaemia was categorised prospectively using the Wifl scale,^{17,18} measuring all three modalities (ankle brachial index, absolute ankle pressure, and toe pressure/TcPO₂) in each patient. For simplicity, throughout this report the Wifl score for ischaemia is referenced according to the ankle brachial index (ABI): Grade 1 – ABI = .6–.79, Grade 2 – ABI = .4–.59, Grade 3 – ABI < .4, even though all three Wifl parameters for ischaemia were used to categorise it. No subjects were Grade 0.

The clinical covariables listed in Table 1 were extracted from the electronic medical records prior to surgery. Antiplatelet therapy was defined as the current use of any one or combination of aspirin, thienopyridine drug, or dipyridamole. Operative variables included the length of the venous conduit, the location of the distal anastomosis, and the minimum diameter of the distended vein before implantation. These data were derived from a

Table 1. Baseline and surgery characteristics in 142 PAOD patients

Characteristic	Overall (n = 142)	Percent or interquartile range
Follow up, median days	480	171–903
Follow up index	.68	.5–1.0
Age, median years	65.5	60–70
White race	129	90.8%
Male sex	129	90.8%
Current smoker	71	50%
Diabetes	67	47.2%
Conduit using GSV	136	95.8%
Length of conduit, median cm	46	37–55
Min vein diam, median mm	4	3.5–4.5
<i>Distal anastomosis</i>		
Popliteal	56	39.4%
Tibial	86	60.6%
Critical limb ischaemia (Fontaine III or IV)	103	72.5%
Wifl Ischaemia Score 2 or 3 (ABI < .6)	105	73.9%
Statin therapy	116	82.3%
Anti-platelet therapy	129	90.8%
Pre-op anti-PC IgM, median units/mL	48.98	32.2–107.9
Pre-op IL-6, median pg/mL	7.2	1.6–20.9
Pre-op C-reactive protein, median mg/L	17.2	5.2–55.4

GSV = greater saphenous vein; ABI = ankle brachial index; PAOD = peripheral arterial occlusive disease; Wifl = wound, ischaemia, foot infection; PC = phosphorylcholine; IL = interleukin.

contemporaneous survey completed by the surgeon. The methods for harvesting the donor vein were left to the discretion of the surgeon (see Results), although all surgeons used the recognised standards of practice, avoiding excessive dissection of the vein and overdilatation. Endoscopic harvesting techniques were not used.

Statistical design

Previous clinical studies have indicated that the lowest levels of anti-PC IgM are closely linked to adverse cardiovascular outcomes, while a much broader range of average or high levels is associated with freedom from risk.^{8–10} Thus, for this study it was chosen, in advance, to compare the cohort of patients in the lowest quartile of anti-PC IgM with those in the highest three quartiles. Based on the median graft survival times from pilot studies,¹³ it was calculated that a total sample size of 108 patients was required to reject the null hypothesis that the graft survivals of the lowest quartile and highest three quartiles are equal, with a probability (power) of .8 and a Type I error of .05.

Statistical analyses were performed using the IBM SPSS software (version 24, Armonk, NY). Group categories were compared using the Pearson chi-square and *t* tests. Hazard ratios for loss of primary patency were estimated by univariable and multivariable Cox proportional hazards tests and survival curves. Plasma cytokine values were log

transformed for analysis, when they were not normally distributed. The defining level for the lowest quartile of IL-6 was 1.6 pg/mL, and for CRP 5 mg/L. Missing values for cytokine and IgM levels were handled by pairwise deletion.

Plasma measurements

Blood samples were obtained pre-operatively, on post-operative day 1, days 3–5, then at post-operative months 1, 3, and 6. Standard assays were employed (see [Supplementary materials](#)).

RESULTS

Characteristics of study population

One hundred and eighty-six consecutive subjects were recruited pre-operatively, and 44 were excluded by the post-operative exclusion criteria (e.g. prosthetic graft). One hundred and forty-two eligible subjects were followed for an average of 1.8 years (1 month–7.4 years). [Table 1](#) summarises the clinical characteristics of this population, their operations, and follow up indices. Sixty-one percent of the bypasses were made to the tibial arteries (39% popliteal), and 96% used the greater saphenous vein (4% arm veins). The surgical harvesting technique was resection and re-implantation in all but three cases, where the in situ technique was used. During their post-operative hospitalisation, 72% of patients received subcutaneous, prophylactic doses of heparin. Regarding cytokine and IgM values, no pre-operative values were missing, but 5–7% of post-operative values were missing. These were missing completely at random (MCAR) in relation to IgM level or outcome, and were handled by pairwise deletion in statistical analyses.

Associations between IgM levels and clinical characteristics

[Table 2](#) compares the main clinical characteristics and follow up parameters for the lowest quartile of pre-operative anti-PC IgM versus those in the higher three quartiles. There was a higher proportion of tibial bypasses in the high three quartile cohort, but otherwise there were no significant differences in the distribution of characteristics between the two groups.

Because the pre-operative clinical conditions of the patient (e.g. diabetes, Fontaine stage, smoking) might influence the baseline IgM level, these associations were examined by univariable linear regressions, and then by multiple stepwise regression ([Table S1](#)). This revealed that only a patient's clinical indication for surgery (i.e. claudication vs. critical limb ischaemia, Fontaine II vs. III–IV) bore a significant association with the pre-operative IgM level, albeit with weak effect ($R^2 = .04$, $F(1,141) = 7.18$, $p < .01$). More severe clinical presentations tended to have lower IgM levels. Thus, this variable was included in subsequent analyses of the relationship between IgM level and graft survival.

Table 2. Characteristics of subjects in the lowest versus highest three quartiles of anti-PC IgM

Characteristic	Lowest quartile		Highest three quartiles		p value ^a
	Number or value	Percent or IQR	Number or Value	Percent or IQR	
Pre-operative anti-PC IgM, median units/mL	23.5	18.1–27.7	61.1	40.7–137.9	
Follow up index, median	.826	.37–1.0	.663	.51–1.0	.88
Pre-operative IL-6, median pg/mL	14.9	1.8–30.1	6.6	1.5–20.2	.1
Pre-operative CRP, median mg/L	21.4	9.2–47	15.7	4–56.4	.53
Age, median	68	62–75	65.3	60–69	.12
White race	29	87.9%	100	91.7%	.31
Male sex	30	90.9%	99	90.8%	.99
Current smoker	13	39.4%	58	53.2%	.16
Diabetes	18	54.5%	49	45.0%	.33
Length of conduit, median cm	47.6	38–60	45.5	37–51	.73
Min vein diam, mean mm	3.9	3.5–4	4	3.5–4.5	.56
<i>Distal anastomosis</i>					
Popliteal	19	57.6%	37	33.9%	.026
Tibial	14	42.4%	72	66.1%	
Critical limb ischaemia (Fontaine III or IV)	27	81.8%	76	69.7%	.17
Wifl Ischaemia Score 2 or 3 (ABI < .6)	25	75.8%	80	73.4%	.79
Statin therapy	28	84.8%	88	81.5%	.66
Anti-platelet therapy	30	90.9%	99	90.8%	.99

ABI = ankle brachial index; CRP = C-reactive protein; IQR = Interquartile range; Wifl = wound, ischaemia, foot infection; PC = phosphorylcholine; IL = interleukin.

^a p values determined by Pearson chi-square or t test.

Clinical and biological factors associated with primary graft failure

Primary endpoints occurred in 50 subjects (35.2%, including 12 graft thromboses and 38 interventions for stenosis). Their median time to failure was 181 days. Because many confounding factors may influence graft patency, each of the potential clinical and biochemical variables that might be associated with graft patency was examined individually, using univariable Cox proportional hazards analysis.

Fig. 1 shows the leading factors that were significantly associated with primary graft failure in a univariable fashion ($p < .05$): the location of the distal anastomosis, the diameter of the arterialised conduit, the lowest quartile of pre-operative anti-PC IgM levels, and the degree of ischaemia as classified by the Wifl scale. Borderline associations with graft survival (p between .05 and .2) were found for: Fontaine Class, conduit length, smoking status, and a pre-operative CRP > 5 mg/L. Age, diabetes, and pre-operative IL-6 levels > 1.6 pg/mL were not associated with graft failure.

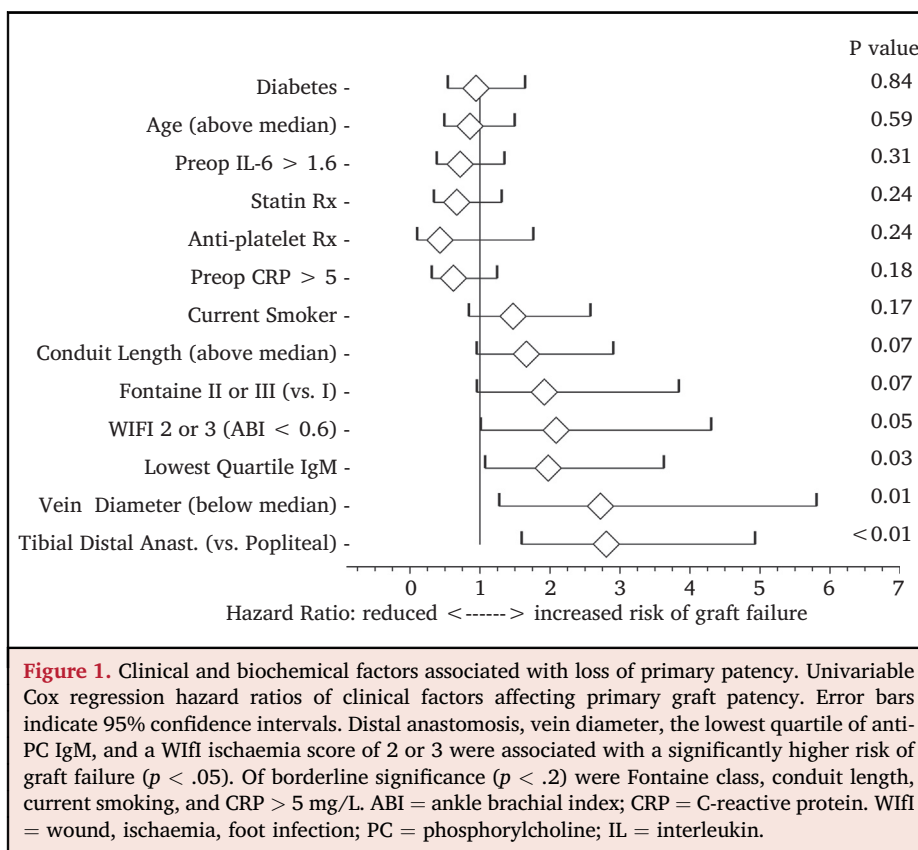
The eight variables found to be individually associated with graft survival at a level of $p = .2$ or less were then included in a Cox multivariable model. Backwards stepwise regressions yielded the key significant factors associated with graft survival. Table 3 shows the final analysis: a patient with an anti-PC IgM level in the lowest quartile experienced a twofold greater risk of graft failure, even after accounting for the distal anastomosis, conduit diameter, smoking, and the Wifl ischaemia category. Fig. 2 compares the Cox model of graft survival of the lowest and highest three quartiles of anti-PC IgM, after accounting for the other confounding variables in Table 3.

Peri-operative dynamics of IgM levels and the inflammatory response

IgM levels fell in concert with the acute rise in systemic inflammation provoked by the surgery. Figure three illustrates these changes and compares the change in IgM levels with the corresponding changes in two classical measures of acute inflammation, interleukin 6 (IL-6) and C-reactive protein (CRP). There was also interest in whether the patients with the lowest levels of anti-PC IgM might exhibit different patterns of post-operative inflammatory response. The post-operative fluctuations in IL-6 and CRP levels were no different between the cohorts with the lowest quartile of pre-operative IgM levels versus the highest three quartiles. As previously noted in Table 3, there was no significant association between the pre-operative levels of anti-PC IgM and those of IL-6 or CRP.

DISCUSSION

This longitudinal, prospective study of 142 patients shows that low plasma levels of a naturally occurring anti-inflammatory factor, anti-phosphorylcholine IgM, have a strong relationship with bypass graft failure, even after accounting for other clinical, biochemical, and technical factors. Those patients in the lowest quartile of pre-operative IgM levels had a twofold higher risk of primary graft failure, controlling for the other significant factors of distal anastomosis, conduit diameter, smoking, and the severity of leg ischaemia. The clinical characteristics of the two groups were generally similar, although the highest three quartiles group had a smaller proportion of popliteal bypasses. Because femoropopliteal bypasses generally have a superior patency to tibial bypasses, this inequality in



distribution would favour an improved graft survival in the low IgM group, whereas the opposite was found in this study. The effects of anti-PC IgM levels observed here support those of prior studies that suggest that a wide range of normal or high levels of this anti-inflammatory antibody may be protective against cardiovascular complications, whereas low levels are associated with higher risk. Supporting this, no significant differences were found in graft survival between those subjects in the second, third, or highest quartiles (not shown).

In the population studied here, the rate of primary graft failure (35.2%) was within the range reported in the literature (25–35%).¹⁹ Also, the other clinical characteristics found to be associated with stenosis and graft failure (graft

diameter, distal anastomosis, smoking, ischaemia category, Fontaine/Rutherford class) have been well documented as risk factors in previous studies of graft patency.^{20–24}

Patients with peripheral arterial disease are well known to have significantly higher levels of chronic systemic inflammation, compared with those without arterial disease.^{25–27} An unregulated inflammatory response to injury has long been implicated as a culprit in the process of intimal hyperplasia and its range of manifestations: from focal, stenotic lesions to a more generalised failure of adaptive remodelling.^{19,28} The patients in this study experienced a typical inflammatory response after surgery, as reflected by increases in IL-6 and CRP, while anti-PC IgM levels fell (Fig. 3). But the post-operative inflammatory response did not appear to be influenced by initial levels of anti-PC IgM (Fig. 4). Nor were the high pre-operative plasma levels of IL-6 and CRP predictive of subsequent graft failure (see Fig. 3).

Indeed, few studies have succeeded in identifying any baseline inflammatory marker that might accurately predict the patency of a peripheral arterial bypass. Gasper and colleagues observed that higher baseline levels of CRP were associated with impaired luminal remodelling²⁹ in humans, but did not correlate this with outcomes. They also observed a higher rate of graft failure in women with baseline CRP >5 mg/L, but not in men.³⁰ The present study also found no association between baseline CRP or IL-6 and later graft failure in the predominantly male population. Stone and colleagues found an association between two markers (CRP and brain natriuretic protein), and overall adverse outcomes after peripheral endovascular

Table 3. Clinical and biochemical factors associated with loss of primary graft patency

Clinical factor	Loss of primary graft patency		
	Hazard ratio ^a	95% CI	p
Tibial distal anast. (vs. popliteal)	2.40	1.35–4.27	0.003
Vein diameter (below median)	2.53	1.18–5.43	0.02
Lowest quartile IgM	2.11	1.09–4.07	0.03
Current smoker	1.82	1.01–3.29	0.05
Wifi Ischaemia Score 2 or 3 (ABI < .6)	2.03	0.96–4.27	0.06

ABI = ankle brachial index; CI = confidence interval; Wifi = wound, ischaemia, foot infection.

^a Multivariable Cox Proportional Hazards.

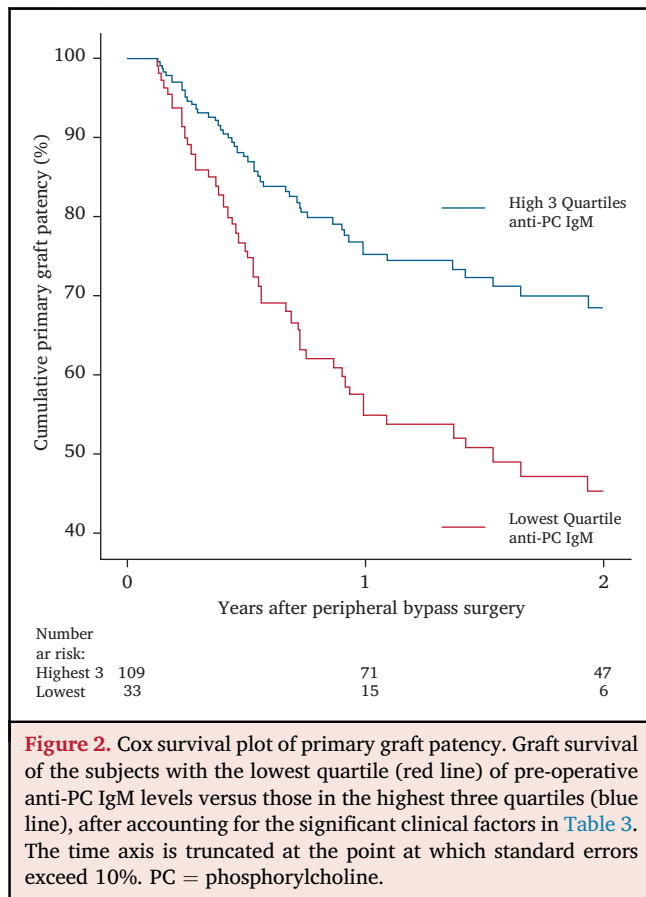


Figure 2. Cox survival plot of primary graft patency. Graft survival of the subjects with the lowest quartile (red line) of pre-operative anti-PC IgM levels versus those in the highest three quartiles (blue line), after accounting for the significant clinical factors in Table 3. The time axis is truncated at the point at which standard errors exceed 10%. PC = phosphorylcholine.

interventions, but no relationship with patency.³¹ In a recent study of gene activation and proteomic changes after peripheral endovascular procedures, DeSart et al. further expanded the list of potentially predictive markers, implicating more complex patterns of proteomic and genetic changes associated with the failure of vascular interventions.³² Taken together, these data suggest that the pro-inflammatory milieu in the post-operative period is more complex than can be accounted for by one or two markers.

Because the levels of this innate anti-inflammatory antibody did not appear to modulate the acute systemic inflammatory response to vascular surgery (as least as reflected by these markers), one might speculate that the anti-inflammatory actions of anti-PC IgM could result from local actions within the vessel wall where this danger associated molecular pattern (phosphorylcholine) is exposed by injured and apoptotic cells. The consistent drop in plasma anti-PC IgM levels seen early post-operatively (Fig. 3) may be caused at least in part, by the consumption of IgM by tissues exposing phosphorylcholine.

This current study has several limitations. The study population consisted predominantly of white males, so no conclusions can be drawn about women, or other races. The present findings are not applicable to endovascular interventions or prosthetic grafts, which were not studied. Because this was an observational study, surgical techniques and post-operative antithrombotic therapy were not standardised.

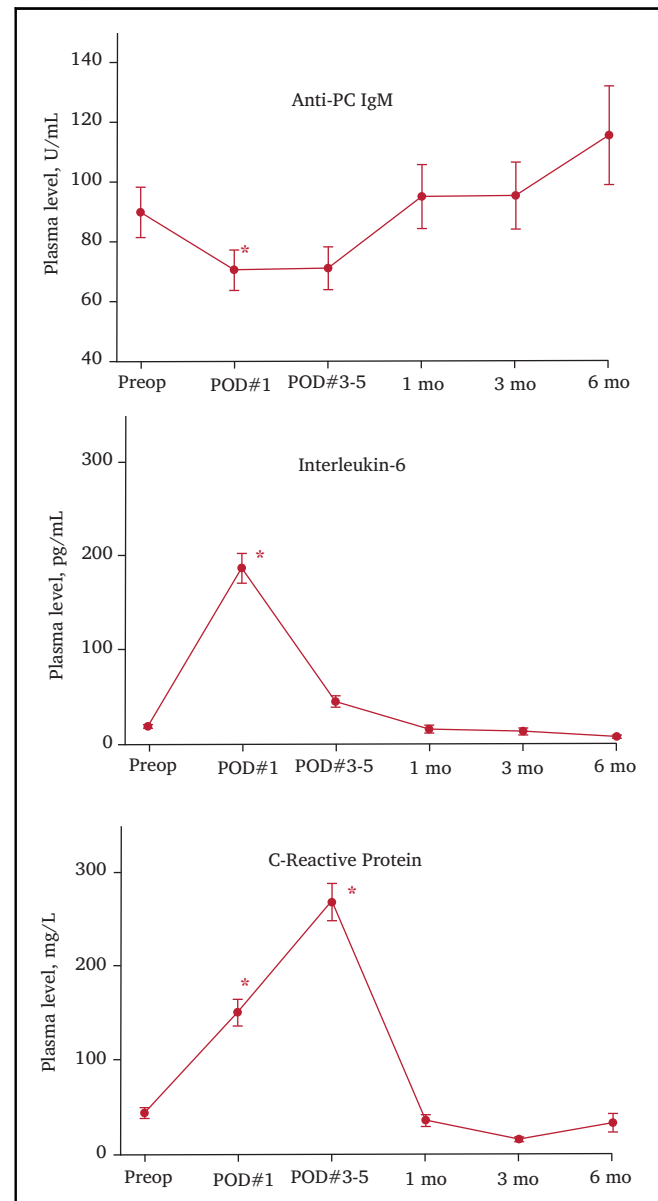
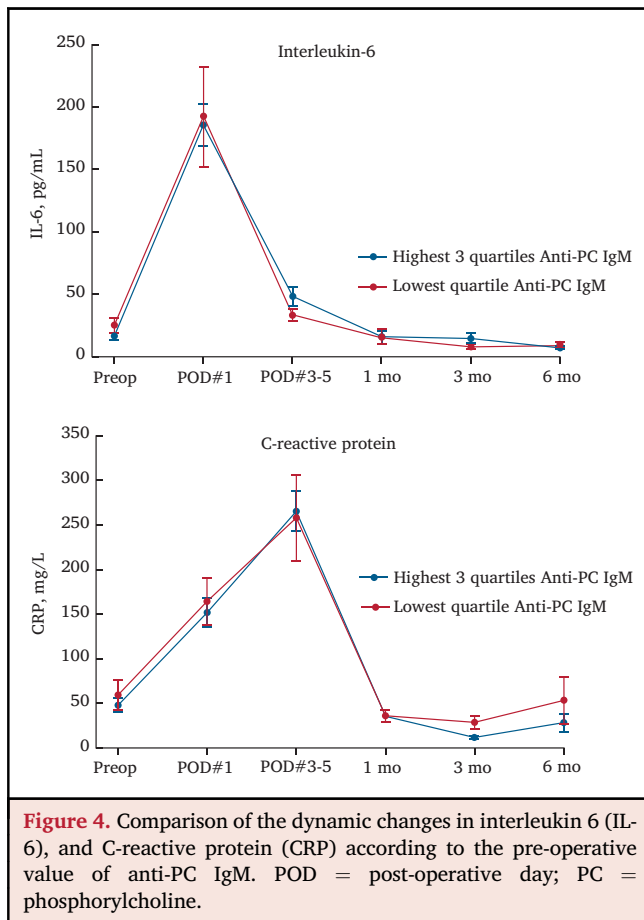


Figure 3. Time course of dynamic changes in anti-PC IgM, interleukin 6 (IL-6), and C-reactive protein (CRP). * $p < .05$, t test comparing post-operative values with the pre-operative baseline. POD = post-operative day; mo = month; PC = phosphorylcholine.

However, the surgical characteristics of the procedures and their post-operative care were relatively uniform: 96% greater saphenous, 98% resected and re-implanted. Of the patients in the study, 80–90% were under treatment with a statin and an antiplatelet agent, and three quarters received post-operative prophylactic doses of heparin. Confident discrimination of the effects of these factors could not be made because of the small numbers of patients who were not receiving aspirin or a statin, for example.

Finally, there is an inherent bias in the late (3–6 month) measurements of IgM and the cytokines. The protocols did not measure cytokines and IgM levels after subjects suffered primary graft failure (median time to failure ~ 6 months), because it was felt that the measurements would



be confounded by the events of thrombosis and/or re-intervention. Thus, the late time points of three and six months in Fig. 3 reflect the attrition of those with failed grafts. However, the earlier time points in the acute peri-operative period are more complete, and representative of the entire cohort.

This current study emphasises the potential value of innate anti-inflammatory mechanisms such as the anti-PC IgM antibody, which acts through a variety of natural anti-inflammatory mechanisms, including the clearance of oxidised LDL and damaged cells, and recruitment of classical complement pathways.^{6,7,11,33} Current guidelines of the European Society for Vascular Surgery¹⁷ recommend single agent antiplatelet therapy after surgical revascularisation, and this current study should not change that. However, if future studies confirm this association between low levels of anti-PC IgM and graft failure, then pre-operative diagnostic testing could identify those who might benefit from closer surveillance and/or more intensive antithrombotic therapy. In the future, therapy via passive or adaptive immunity may also be possible.

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CONFLICT OF INTEREST

None.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejvs.2018.09.010>.

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