Local Perivascular Adiponectin Associates with Lower Extremity Vascular Surgery Wound Complications*

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Abstract

Background. Wound complication rates after lower extremity vascular surgery procedures stand as high as 40%, and represent a major cause of morbidity, mortality, and cost. In view of increasing recognition of adipose tissue involvement in homeostasis and the response to injury, we hypothesized that adipose phenotype links to surgical wound outcomes.

Methods. Clinical history, peripheral blood, and subcutaneous and perivascular adipose tissue were prospectively collected at the time of surgery in patients undergoing lower extremity revascularization (LER) and lower extremity amputations (AMP). Nine biologic mediators [adiponectin, interleukin (IL)-1β, IL-6, IL-8, leptin, monocyte chemoattractant protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, resistin, and tumor necrosis factor (TNF)] were assayed in the adipose tissues and plasma. 30-Day wound complications were captured real time. Logarithmic transformation of mediator levels was performed based on positively skewed, non-Gaussian distribution and data were compared using the Student's t-test. Bonferroni correction was used for multiple comparisons.

Results. Sixty-six patients undergoing LER or AMP for severe PAD were enrolled. The 30-day follow-up rate was 92.4%. In total, 19 (29%) patients developed wound complications. Patients who developed wound complications had elevated perivascular adiponectin levels (mean$\pm$standard error, 2372.45$\pm$648.64ng/ml versus 832.53$\pm$180.54ng/ml, p=0.004). Perivascular IL-1β levels were lower among patients with wound dehiscence (0.41$\pm$0.004pg/ml versus 0.73$\pm$0.09pg/ml, p=0.001).

Conclusions. Local adipose tissue mediator levels at the time of operation demonstrate a previously un-described compartment-specific relationship to wound outcomes in patients undergoing lower extremity vascular surgical procedures. These associations provide fertile directions for defining the mechanisms underlying the pathogenesis of wound complications and

their prevention.

INTRODUCTION

The incidence of wound complications after lower extremity vascular surgical procedures remains high, approaching 30% to 40% even in modern series.[1-4] The association of wound complications with bypass graft failure, limb loss, revision of amputation to higher levels, increased length of stay, hospital readmission, cost, and mortality has been extensively demonstrated.[3, 5-11] However the biologic mechanisms underlying wound complications remain incompletely understood.

Once thought to serve solely as a structural connective tissue, adipose is increasingly recognized for its active role in cell-cell signaling, modulation of vascular smooth muscle function, remodeling, and inflammation—all components of wound healing.[12] Obesity has been linked to postoperative wound complications and circulating adipokine levels have been associated with surgical site infections (SSI) after certain gastrointestinal procedures.[10,13-19] However the impact of the local adipose microenvironment on wound outcomes has not been explored despite this being the dominant tissue in the operative field.

Theorizing complex signaling interplay among local adipose tissue and the adjacent surgical site, we investigated our hypothesis that specific local mediators link to 30-day wound complications in patients undergoing open lower extremity vascular surgical procedures for peripheral arterial disease (PAD).

METHODS

Study Participants and Data Collection. Patients undergoing infrainguinal, lower extremity revascularization (LER) or amputation (AMP) for severe PAD (both lifestyle limiting claudication failing non-operative therapy and critical limb ischemia) at a single institution between January 1, 2013 and June 17, 2015 provided written informed consent for prospective collection of demographic and clinical data under an Institutional Review Board (IRB)-approved protocol. Exclusion criteria included age less than 18 years, emergent indication for revascularization or amputation, participation in another clinical interventional research study, and intervention for any reason other than advanced chronic peripheral arterial occlusive disease.

Sample Procurement and Protein Assay. Methods for protein assay from subcutaneous and perivascular adipose tissues have been described previously.[20] Briefly, at the time of surgery, peripheral blood, subcutaneous adipose tissue, and perivascular adipose tissue were obtained by surgeons who had been briefed on standardized sampling techniques. Fifteen milliliters of peripheral blood were obtained at the time of peripheral intravenous line placement and plasma was isolated by centrifugation for 15 minutes at 2000g at room temperature. Surgeons collected 50 to 500 milligrams of white adipose tissue from each of two locations: (1) subcutaneous tissue at the site of incision, and (2) perivascular tissue contiguous to the adventitia of diseased segments of the femoral, popliteal, or crural arteries. These are samples of white adipose tissue based on largely negative uncoupling protein-1 (UCP-1) staining in a separate cohort of patients (unpublished data). All samples were immediately flash frozen in liquid nitrogen then stored at -80^{o}C until the time of analysis. Proteins (both intracellular and extracellular) were isolated from the samples using ice-cold Dulbecco's phosphate-buffered saline with protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). This solution was then homogenized and centrifuged

(2,000*g* x 5 minutes) to remove gross debris. The supernatant was again centrifuged (10,000*g* x 10 minutes). The supernatant was then collected for quantitative protein analysis using a Luminex multiple antigen flow microparticle bead assay (Luminex Corporation, Austin, TX). Based on previous literature, nine key biologic mediators were assayed: adiponectin, interleukin (IL)-1β, IL-6, IL-8, leptin, monocyte chemoattractant protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, resistin, and tumor necrosis factor (TNF).[21-39] Intra-assay coefficient of variability is 2-16% and accuracy is 75-100% for the aforementioned proteins using the Luminex system. Quantities were adjusted by the total initial fat tissue mass of each sample.

Outcome Variables. The primary endpoint of our study was the development of wound complications, defined as superficial incisional SSI, deep incisional SSI, dehiscence, or other (seroma, lymphocele, hematoma), according to previously published criteria.[4] Wounds were assessed during the index hospitalization and regularly scheduled outpatient follow-up, as well as during any other emergency department, clinic, or inpatient encounters during which the patient presented with complaints related to the index operation. Due to the variable enrollment times of patients over the two year study period, patients were censored at 30 days postoperatively.

Statistical Analysis. Preliminary evaluation of the mediator concentration data set revealed a non-Gaussian, right-skewed distribution, which was normalized using a logarithmic (base 10) transformation, confirmed by Shapiro-Wilk testing. Continuous data were analyzed using Student's t-test based on normality of distribution. Bonferroni correction was used for multiple comparisons. All statistical analyses were conducted using SAS software, v9.3 (SAS Institute, Inc., Cary, NC.)

RESULTS

Baseline Patient Characteristics. Forty-four patients undergoing LER and 22 undergoing AMP for severe PAD were enrolled. Sixty one of 66 (92.4%) patients remained enrolled through the 30-day follow-up period. Of the 5 who did not complete 30-day follow-up, 3 expired (2 during the index hospitalization due to aspiration and cardiac arrest, respectively, and 1 after being readmitted with pulmonary edema in the setting of heart failure) and 2 were lost to follow-up. In total, 19 (31%) patients in our cohort developed wound complications. Baseline demographic and clinical characteristics of those who developed wound complications versus those who did not were similar (Table I). Mean patient age was in the late seventh decade. Thirty nine percent of women developed wound complications versus 26% of men, but this difference was not statistically significant. The majority of both groups were Caucasian, were former or current smokers, had comorbid diabetes, renal, and cardiovascular disease, and were on anti-platelet and statin therapy. Approximately half were on angiotensin converting enzyme inhibitors or angiotensin receptor blockers. Mean serum high density lipoprotein levels were low, but total cholesterol and low density lipoprotein were normal, and body mass index was in the overweight range (Table I).

Association of Local Mediator Levels and Wound Complications. Among wound complications, superficial surgical site infections predominated (Table II). Patients who developed wound complications had elevated perivascular adiponectin levels compared to those who did not (mean$\pm$standard error, 2372.45$\pm$648.64ng/ml versus 832.53$\pm$180.54ng/ml, p=0.004) (Table III, Supplemental Table I.) This association was robust, remaining statistically significant after Bonferroni adjustment (α/9=0.006). There was also a trend toward elevated perivascular and subcutaneous leptin levels in patients who developed wound complications, but this was not

significant after Bonferroni correction (Table III, Supplemental Table I-II.) Subgroup analysis revealed that perivascular IL-1β levels were higher among patients with wound dehiscence, although the 30-day event rate was low (4 patients; Table III, Supplemental Table IV.) There was no relationship between the levels of these mediators in plasma and wound complications (Table III; Supplemental Table II-III, V-VI) in this relatively small cohort of patients.

DISCUSSION

Adipose holds the largest tissue mass locally in a surgical wound, and we have previously shown that the typical focal trauma inflicted at the time of surgery greatly perturbs the biology of this local white adipose.[40] We have also recently discovered that focal surgical trauma induces adipose browning even at sites remote from the operation.[41] In the current report we now link baseline white adipose phenotype to a benchmark surgical outcome—wound complications. Here, we show that elevated adiponectin and decreased IL-1β expression in the local perivascular tissue at the time of operation associate with the development of wound complications in the early, 30-day period following surgery. These differences were highly significant, surviving Bonferroni correction for multiple testing, whereas clinical and demographic differences were not; this may suggest a larger effect size relating local mediator concentrations and the development of wound complications. In addition, this differential expression profile was compartment specific: it was only present in the perivascular compartment. Patients with and without wound complications had similar circulating mediator levels, but had significantly different adiponectin and IL-1β expression in tissue contiguous to the diseased artery. There were several other adipose-related biomarker trends between these small cohorts, further supporting potential signaling pathways between the vasculature and these

tissues. Note that we index the mediator of interest to local tissue volume since we believe that local paracrine signaling (not total body levels, for example) is linked to clinical events such as wound complications. Using total protein as the denominator for such analytic approaches is prone to confounding effects of blood contamination and perturbations of other greatly expressed proteins that then skew the results for the mediator of interest. In fact, such a data manipulation dissolved the observed relationships.

While novel, these unexpected findings are consistent with and build upon previous reports by the authors and others. We have previously shown that elevated perivascular adiponectin was associated with symptomatic status (a clinical marker of destabilized atheromatous plaque) in patients undergoing carotid endarterectomy, and that a similar trend existed with perivascular IL-1β.[42] We also recently demonstrated that local adipose adiponectin was inversely correlated with eventual flow volume (a marker of arteriovenous fistula maturation) in the early stages following hemodialysis access creation surgery.[43] Investigators studying circulating plasma adipocytokine levels found a nearly significant trend toward elevated preoperative leptin in patients who developed SSI.[17, 18] A similar correlation between circulating adiponectin and wound infection was not found.[18] Others have shown that elevated preoperative circulating adiponectin was significantly associated with the development of postoperative infection, although these authors did not disaggregate SSI and remote infections.[16] To date, however, links between *local* adipose tissue and wound complications have not yet been reported despite adipose standing as the dominant tissue volume in the surgical wound (and thus a potential contributor to wound events via paracrine signaling).

Here a seemingly paradoxical relationship was found between local perivascular adiponectin—traditionally viewed as a protective vascular mediator—and wound outcomes. This

may suggest disparate roles for local versus systemic adiponectin, or undefined effects of adiponectin. For example, adiponectin has been shown to modulate macrophage polarization, reducing reactive oxygen species and related gene expression in a murine model.[44] Although inflammatory cells have long been known to be necessary constituents for local wound healing, recent work in humans has suggested that an exaggerated local cytokine expression is associated with poor wound healing.[45] Thus elevated local adiponectin may be an adaptive response to a hyper-inflammatory local milieu in patients who are at higher risk for wound complications.

Our findings must be interpreted within the context of the study design. Given our relatively small sample, subtle differences in protein levels can be missed (type II error); the interesting trends noted in the other mediators may be biologically noteworthy. Type I error was in part addressed by Bonferroni correction, but other factors such as the precision and accuracy of the Luminex assay system and sampling error could also lead to type I errors. Multivariate analysis to control for clinical factors was considered. However, given our event rate of 19 wound complications in our sample of 61 patients with follow-up, inclusion of only 1-2 predictors would be possible to maintain the event per predictor variable ratio >10 to avoid over-fitting our logistic regression model.

Certainly the post-surgical response of the local adipose tissue depots to the focal trauma would be relevant, but such longitudinal sampling studies are not feasible in humans. Nearly all of our patients were Caucasian, which may limit the generalizability of our results. As with other studies of tissue mediators, there is always risk of exposure misclassification from cross-contamination of perivascular tissue with whole blood, subcutaneous adipose, or other tissues. Furthermore, wound complications require clinicians' assessment of the wound, allowing a measure of subjectivity which may have resulted in outcome misclassification in some cases.

Most importantly, precise mechanisms cannot be derived from this observational data, though the novel associations discovered should spur such important studies. The current report was designed as an exploratory venture to test for links between adipose and surgical outcomes; certainly further investigation will be needed to definitively understand these heretofore un-described relationships.

In conclusion, levels of local mediators at the time of operation demonstrate compartment-specific relationships to wound outcomes in patients undergoing lower extremity vascular surgical for PAD. These associations likely have implications for mechanisms underlying the pathogenesis of healing and wound complications, and in the future may suggest novel interventional strategies to reduce wound complications based on the plasticity of the adipose organ's phenotype[46-49].

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TABLES AND FIGURE LEGENDS

Table I. Baseline Patient Characteristics.

Variable	No Wound Complication (n=42)	Wound Complication (n=19)	P value
Mean age, years (SD)	69.4 (11)	66.2 (11)	0.28
Sex			
Male (%)	28 (67)	10 (53)	0.29
Female (%)	14 (33)	9 (47)	
Race			
White (%)	33 (79)	12 (63)	0.33
Hispanic (%)	3 (7)	5 (26)	
Black (%)	3 (7)	1 (5)	
Other (%)	3 (7)	1 (5)	

Mean BMI (SD)	27.3 (6.0)	29.5 (6.9)	0.36
Comorbidities			
Prior stroke (%)	20 (48)	9 (47)	0.99
Prior MI (%)	19 (45)	11 (58)	0.36
Heart failure (%)	18 (43)	11 (58)	0.28
HTN (%)	41 (98)	17 (89)	0.17
Hyperlipidemia (%)	37 (88)	18 (95)	0.42
Diabetes mellitus (%)	34 (81)	15 (79)	0.85
COPD (%)	18 (43)	10 (53)	0.48
Renal insufficiency (%)	8 (19)	6 (32)	0.45
Smoking status			
Never	6 (14)	5 (26)	0.48
Former	23 (55)	8 (42)	
Current	13 (31)	6 (32)	
Preoperative medications			
Aspirin (%)	26 (62)	12 (63)	0.93
Clopidogrel (%)	22 (52)	9 (47)	0.72
Statin (%)	27 (64)	13 (68)	0.75
Beta blocker (%)	23 (55)	9 (47)	0.59
ACE-inhibitor (%)	21 (50)	9 (47)	0.85
ARB (%)	24 (57)	10 (53)	0.74
Preoperative laboratory values			
Mean total cholesterol, mg/dl (SD)	153 (51)	165 (35)	0.46
Mean HDL, mg/dl (SD)	42 (18)	46 (11)	0.46
Mean LDL, mg/dl (SD)	73 (38)	94 (32)	0.10
Mean triglycerides, mg/dl (SD)	190 (145)	152 (101)	0.40
Mean CRP , mg/L (SD)	45 (67)	80 (102)	0.30
Mean albumin, g/dl (SD)	3.7 (0.8)	3.6 (0.7)	0.71
Procedure			
Infrainguinal revascularization (%)	28 (67)	13 (68)	0.89
Lower extremity amputation (%)	14 (33)	6 (32)	

SD, standard deviation; BMI, body mass index; MI, myocardial infarction; HTN, hypertension; COPD, chronic obstructive pulmonary disease; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; Cr, creatinine

Table II. 30-Day Postoperative Wound Complications.

Variable	Total cohort (n=61)
Wound complications	
Superficial SSI (%)	12 (20)
Deep SSI (%)	1 (1.6)
Dehiscence (%)	4 (6.6)
Other (%)	3 (4.9)
Any wound complication (%)	19 (31)

SSI, surgical site infection

Table III. Relationship of Mediator Levels in Perivascular, Subcutaneous, and Plasma Compartments and Wound Complications.

	Perivascular			Subcutaneous			Plasma		
Mediator	No Wound complication (n=42)	Wound Complication (n=19)	P value	No wound complication (n=42)	Wound Complication (n=19)	P value	No wound complication (n=42)	Wound Complication (n=19)	P value
Mean log adiponectin, pg/ml (SD)	5.74 (0.35)	6.09 (0.51)	0.004*	5.91 (0.33)	6.09 (0.41)	0.08	7.15 (0.41)	7.39 (0.59)	0.12
Mean log leptin, pg/ml (SD)	2.84 (0.32)	3.07 (0.36)	0.01	2.95 (0.32)	3.23 (0.39)	0.02	3.94 (0.52)	4.04 (0.55)	0.49
	No Dehiscence (n=57)	Dehiscence (n=4)	P value	No Dehiscence (n=57)	Dehiscence (n=4)	P value	No Dehiscence (n=57)	Dehiscence (n=4)	P value
Mean log IL-1β, pg/ml (SD)	-0.24 (0.28)	-0.39 (0.03)	0.001*	-0.29 (0.27)	-0.29 (0.18)	0.98	-0.43 (0.32)	0.04 (0.73)	0.10

Log, logarithm base 10; SD, standard deviation; IL, interleukin

*Statistically significant after Bonferroni correction ($\alpha/9 = 0.05/9 = 0.006$)

Abbreviations: SSI, surgical site infection; PAD, peripheral arterial disease; LER, lower extremity revascularization; AMP, lower extremity amputation; IRB, institutional review board; IL, interleukin; MCP, monocyte chemoattractant protein; PAI, plasminogen activator inhibitor; TNF, tumor necrosis factor