BRIEF REPORT

Circulating PCSK9 is lowered acutely following surgery

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Background: A decrease in serum low-density lipoprotein cholesterol (LDL-C) is well documented after acute stress. Plasma proprotein convertase subtilisin kexin 9 (PCSK9), which promotes degradation of low-density lipoprotein receptor (LDL-R) resulting in reduced plasma clearance of low-density lipoproteins (LDL) and an increase in serum LDL-C, would be predicted to decrease. Yet, a few studies have demonstrated an increase 1-8 days after acute stress. Our objective was to assess the earlier status of plasma PCSK9, within the first 24 hours of onset of stress.

Methods: We measured serum lipids and plasma PCSK9 in 39 patients before and soon after an elective surgical procedure (abdominal aortic aneurysm (AAA) repair).

Results: We observed an early decrease in PCSK9 following surgery, as well as a decrease in total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), non-high-density lipoprotein cholesterol (non-HDL-C) and triglycerides (TG).

Conclusion: Unlike other studies which showed an increase in PCSK9 after the onset of stress, our study detected a fall in PCSK9 following acute surgical stress. The observed difference is likely due to the earlier timing of PCSK9 measurement in our study. Further studies involving serial poststress measurements for several days are needed to determine whether PCSK9 behaves as an acute-phase reactant, whether it displays a biphasic response to acute stress, and whether changes in circulating PCSK9 are responsible for lipoprotein changes observed after surgical stress. (Clinical Trial Registration: ClinicalTrials.gov study ID NCT00493389)

KEYWORDS

acute stress, lipoproteins, PCSK9, Surgery

1 | INTRODUCTION

It is well recognized that changes in serum lipid levels occur with acute stress. These have been reported with acute myocardial infarction (MI),¹ stroke,² acute infection,³ and low- and intermediate-risk surgery.⁴ The major change is a decrease in low-density lipoprotein cholesterol (LDL-C) levels. This occurs even in patients with heterozygous familial hypercholesterolemia following surgery.⁵ The factors that mediate these changes remain unclear. Suggested candidates include catecholamines, cytokines, and other molecules such as insulin and glucagon that regulate the levels of the low-density lipoprotein

receptor (LDL-R).⁶ It is not known whether proprotein convertase subtilisin kexin 9 (PCSK9) plays a role.

Following its initial discovery in 2003 by Seidah et al as a neural differentiation factor,⁷ PCSK9's role in lipoprotein metabolism was demonstrated by the association of 2 single nucleotide mutations in the *PCSK9* gene with autosomal dominant hypercholesterolemia.⁸ Overexpression of PCSK9 in mice resulted in a dramatic reduction in LDL-R protein, but not mRNA, suggesting that PCSK9 accelerated degradation of the LDL-R, resulting in hypercholesterolemia.⁹⁻¹¹ Knockout of *PCSK9* in mice resulted in an increase in LDL-R protein but not mRNA, leading to increased clearance of LDL and a

decrease in serum cholesterol levels.¹² Secreted PCSK9 binds to the EGF-like repeat (EGF-A) domain of the LDL-R¹⁰ and is endocytosed with the LDL-R, redirecting the LDL-R from the endosomal recycling pathway to the lysosomal compartment for degradation,⁹ thereby elevating circulating LDL-C levels.^{9,12-14} Plasma PCSK9 correlates positively with LDL-C,¹⁵⁻¹⁸ and statin therapy increases circulating PCSK9 levels.^{19,20}

Studies are emerging that suggest a possible link between acute stress and PCSK9. A study in rats showed that stress induced by lipopolysaccharide (LPS) administration, an established model of gram-negative bacteremia, resulted in increased hepatic expression of PCSK9 mRNA in rats.²¹ Another study of acute MI in rats showed an elevation in circulating PCSK9 protein.²² Human studies have also demonstrated that stress from severe trauma²³ and MI^{24,25} raises plasma PCSK9 levels. Elevated plasma PCSK9 would be expected to be associated with elevation in LDL-C. Instead, the observation is that LDL-C decreases after acute stress. We hypothesized that this dichotomy may be due to timing of plasma PCSK9 measurement. In human studies, plasma PCSK9 has been measured 24 hours²⁴ to about 8 days^{23,25} after the onset of stress. Our objective was to determine the status of plasma PCSK9 within the first 24 hours of onset of stress.

2 | MATERIALS AND METHODS

2.1 | Subjects

Forty patients scheduled for elective abdominal aortic aneurysm (AAA) repair surgery were recruited for participation in another study which sought to measure cortisol response to administered adrenocorticotrophic hormone before and after surgery (ACTH; ClinicalTrials.gov study ID NCT00493389, <http://www.clinicaltrials.gov>). The original study was approved by the Ottawa Health Science Network Research Ethics Board (OHSN-REB). As part of the study, consent was provided by the participants for measurement of other blood parameters that might be associated with the stress response, including the ones reported here.

Blood was drawn a few days to a few weeks prior to surgery, before and after the administration of ACTH. For this ancillary study on PCSK9, only the pre-ACTH blood samples were analyzed. In the presurgery test, the patient had fasted for 12 hours prior to the sample being taken. In the postsurgery test, blood was drawn within 12 hours of surgery completion. The patients had fasted in preparation for surgery. The presurgery samples were all taken before 10 AM, and the postsurgery samples were taken between 1 PM on the day of surgery and 6 AM the next day, depending on the time of surgery. During and after surgery, patients received various types of intravenous fluids including blood, Pentaspan, Ringer's lactate and normal saline. None received glucose-containing fluid.

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured in plasma. LDL-C was calculated for each sample using the Friedewald equation. One patient had a TG value of >4.5 mmol/L and LDL-C could not be calculated, and this patient was eliminated from the analysis. Non-HDL cholesterol was calculated by subtracting HDL-C from TC.

Albumin, a well-recognized negative acute-phase reactant that decreases very soon after surgery,²⁶ was also measured, as a positive control of the effect of acute surgical stress.

To account for possible effects of hemodilution or hemoconcentration, all postoperative values were adjusted for hematocrit based on the formula²⁷:

$$\text{Corrected value} = (\text{Post-operative value}) \times \frac{1 - \text{Post-operative hematocrit}}{1 - \text{Pre-operative hematocrit}}$$

2.2 | Assays

To obtain plasma, blood was collected into EDTA Vacutainer tubes and centrifuged at 1000 g for 10 minutes at room temperature.

PCSK9 was measured using a human PCSK9 enzyme-linked immunosorbent assay (ELISA) from CyClex Co (Japan). All samples were assayed a minimum of 2 × with an intra-assay CV of 1.5%-2.6% and an interassay CV of 2.9%-7.1%. Data are shown as the mean of duplicate wells.

2.3 | Statistical analysis

Continuous variables were assessed using mean ± standard deviation (SD). We assessed for normality using the Shapiro-Wilk test. Statistical significance was defined as $P < .05$ (two-sided). Paired

TABLE 1 Baseline demographics of study population

Characteristic	Study Population n = 39
Age - mean (SD)	71.1 (8.29)
Male gender - n (%)	31 (79.5)
BMI - mean (SD)	29.2 (5.55)
Caucasian race - n (%)	37 (94.9)
Comorbid conditions	
Smoking - n (%)	8 (20.5)
Diabetes - n (%)	6 (15.4)
Arthritis - n (%)	7 (7.9)
Medication Use	
Statin - n (%)	32 (82.1)
ASA - n (%)	25 (64.1)
Clopidogrel - n (%)	2 (5.1)
Warfarin - n (%)	2 (5.1)
Beta blocker - n (%)	18 (46.2)
Calcium channel blocker - n (%)	16 (41.0)
ACEi - n (%)	24 (61.5)
ARB - n (%)	4 (10.3)
PPI - n (%)	5 (12.8)
Inhaled corticosteroid - n (%)	2 (5.1)
Insulin - n (%)	0 (0)
Biguanide - n (%)	4 (10.3)
Sulfonylurea - n (%)	2 (5.1)

BMI, body mass index, ASA, acetylsalicylic acid, ACEi, angiotensin-converting enzyme inhibitor, ARB, angiotensin receptor blocker, PPI, proton pump inhibitor.

t test was used to assess the difference between preoperative and postoperative albumin levels (normally distributed), and Wilcoxon matched-pairs signed-rank test was used to assess the difference between preoperative and postoperative levels of parameters that were

not normally distributed (PCSK9, TC, TG, HDL-C, LDL-C, and non-HDL-C). Spearman rank correlation coefficient (*r*) was used to describe the linear correlation between albumin and PCSK9. Statistical analyses were conducted using GraphPad Prism version 5 and IBM SPSS Statistics version 21.

TABLE 2 Mean values pre- and postsurgery

Parameter	Preoperative Mean (SD)	Postoperative Mean (SD)	P-value
Total cholesterol (mmol/L)	3.90 (1.10)	2.78 (0.99)	<.0001
HDL cholesterol (mmol/L)	1.07 (0.33)	0.75 (0.32)	<.0001
LDL cholesterol (mmol/L)	2.11 (0.98)	1.48 (0.86)	<.0001
Non-HDL cholesterol (mmol/L)	2.82 (1.12)	2.02 (0.91)	<.0001
Triglycerides (mmol/L)	1.48 (0.82)	1.15 (0.49)	.0003
PCSK9 (ng/mL)	442.9 (162.9)	262.9 (122.0)	<.0001
Albumin (g/L)	40.1 (2.94)	27.2 (6.79)	<.0001

3 | RESULTS

3.1 | Study population

The baseline demographics, select comorbid conditions, and concurrently used medications of the study population are summarized in Table 1. Thirty-one patients (79.5%) were male and 8 (20.5%) were female. The majority, 95%, were Caucasian. Their mean age at the time of surgery was 71.1 ± 8.29 years, and their mean BMI was 29.2 ± 5.55 . Twenty-nine of the 39 patients (74.3%) underwent open AAA repair and 10 underwent endovascular aneurysm repair. Of note, 32 of 39 patients, or 82.1%, were taking a statin at the time of this study.

FIGURE 1 Waterfall plot demonstrating absolute change for PCSK9 (ng/mL), total cholesterol (TC; mmol/L), LDL cholesterol (LDL-C; mmol/L), HDL cholesterol (HDL-C; mmol/L), non-HDL cholesterol (non-HDL-C; mmol/L), triglycerides (TG; mmol/L), and albumin (Alb; g/L). All data have been arranged in order of most negative to most positive absolute change in PCSK9

3.2 | Lipoprotein response to surgical stress

Table 2 summarizes the measured and calculated lipoprotein values, before and after AAA repair. The postsurgery blood samples were drawn a mean of 6 hours and 34 minutes after the time of induction of anesthesia, which we took as the onset of acute stress. All postsurgery values have been adjusted for hematocrit as described in Section 2.

The change in each lipoprotein category is demonstrated in waterfall plots in Figure 1. There was a statistically significant decrease in TC, LDL-C, HDL-C, non-HDL-C, and TG.

3.3 | PCSK9 response to surgical stress

The mean presurgery serum PCSK9 value was 442.9 ± 162.9 ng/mL, and postsurgery, the mean measured PCSK9 adjusted for hematocrit was 262.9 ± 121.2 ng/mL. The change was statistically significant at $P < .0001$ (Table 2).

When analyzed individually, serum PCSK9 decreased in 30 of 39 patients, did not change in one patient, and increased in 7 of 39 patients postsurgery (Figure 1).

Albumin decreased postsurgery ($P < .05$), which is in keeping with previous studies²⁶ (Table 2, Figure 1). There was a positive correlation between the absolute changes in albumin and PCSK9 ($r = 0.4688$, $P = .0027$).

4 | DISCUSSION

The main finding in our study is an early decrease in plasma PCSK9 in response to surgical stress. As expected, there was also a significant decrease in TC and LDL-C, which is in line with previously published work.^{4,28,29} Published work on the response of circulating PCSK9 to acute stress is limited to measurements made a longer time after the onset of acute stress than in this study. Those studies demonstrated an increase in PCSK9 levels after stress, which is the opposite of our finding. In animal models, Feingold and colleagues showed that injection of LPS into mice led to an increase in hepatic PCSK9 mRNA expression.²¹ Zhang et al induced acute MI in rats with ligation of the left anterior descending artery and found an increase in plasma PCSK9 concentration. They found that the level peaked at 48 hours.²² In human studies, Le Bras and colleagues found a twofold increase in plasma PCSK9 between 36 hours and 8 days after suffering multiple trauma.²³ In patients with heart disease, it was shown that PCSK9 was increased in acute MI, 3–5 days after onset of chest pain.^{24,25} Similarly, in human studies of ischemic stroke, it was found that PCSK9 increased between day 1 and day 7 after the stroke.³⁰

We assessed PCSK9 within 24 hours of onset of stress, a mean of 6.5 hours after the induction of anesthesia, whereas other studies have performed so between 24 hours²⁴ to about 8 days^{23,25} after the onset of stress. To our knowledge, this is the first study to demonstrate a decrease in plasma PCSK9 after acute surgical stress. PCSK9 circulates in 2 major forms, the full-length form and the furin-cleaved truncated form.^{31,32} The ELISA assay from CyClex Co (Japan) used in

our study measured both forms, that is, total PCSK9. The 4 studies in humans mentioned above used either the same CyClex kit or the Quantikine® PCSK9 ELISA kit from R&D Systems, Inc., which also measured total PCSK9. Thus the discrepancy between the decrease in PCSK9 in our study and the increase previously reported does not result from measurement of different forms of PCSK9.

The significant correlation between the absolute changes in PCSK9 and albumin, a well-recognized negative acute-phase reactant that decreases soon after surgery,²⁶ suggests that PCSK9 may also be a negative acute-phase reactant. Our data, when seen against the rest in the literature, raise the possibility of a biphasic PCSK9 response to acute stress. This can be clarified in future studies with serial post-stress measurements. Further studies are also required to demonstrate whether changes in circulating PCSK9 are responsible for lipoprotein changes observed after surgical stress.

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