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Reduced plasma PCSK9 response in patients with bacteremia is associated with mortality

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Running headline up to 30 characters: PCSK9 plasma levels in bacteremia

Abstract

Background: The proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme controls blood cholesterol levels by down-regulating the expression of the low-density lipoprotein receptor (LDLR). Since pathogenic lipids (e.g. LPS) are removed from the bloodstream by an LDLR/PCSK9 dependent mechanism, PCSK9 inhibitors have been suggested to be beneficial also in the treatment of infections. We quantitated the plasma PCSK9 levels in patients with culture-positive bacteremia and explored pathogen and site-of-infection dependent effects as well as correlations between patient characteristics and outcome.

Methods: PCSK9 in the plasma was measured with ELISA from 481 blood culture-positive infection patients on days 0 to 4 after admittance to the emergency department. Patient outcome, the clinical and laboratory data were gathered retrospectively from patient records.

Results: The plasma PCSK9 level was elevated equally in patients with Gram-positive or Gram-negative bacterial infections; especially high levels were seen in patients with a lower respiratory tract infection and *Streptococcus pneumoniae* bacteremia. PCSK9 levels showed a significant positive correlation with CRP. Bacteremia patients with liver disease or a history of abusive alcohol use had significantly lower levels of plasma PCSK9. Reduced PCSK9 plasma responses in patients were significantly associated with day 7, 28 and 90 mortalities.

Conclusion: PCSK9 becomes upregulated in blood culture-positive infections. Plasma PCSK9 resembles acute phase proteins; its expression is induced during an infection, reduced in liver disease and correlates positively with CRP. PCSK9 levels were lower in patients with a fatal prognosis.

Key words: PCSK9; sepsis; bacteremia; CRP

Introduction

The proprotein convertase subtilisin/kexin enzyme 9 (PCSK9) is a serine protease that is preferentially expressed in the liver and known to possess autoproteolytic activity [1-3]. The mature PCSK9 protein serves as a chaperone for cell-surface receptors and escorts them to intracellular endosome/lysosome degradation compartments. The key function of PCSK9 is to upregulate the levels of circulating low-density lipoprotein (LDL) by reducing the expression of the low-density lipoprotein receptor (LDLR) and reducing the clearance of hepatic lipids. Patients with a *PCSK9* loss-of function genotype are healthy, but show exceptionally low levels of LDL and few incidence of coronary heart disease (CHD), which validates PCSK9 as biological target for drug development [4]. The first two PCSK9 targeting monoclonal antibodies, alirocumab and evolocumab, are now being used to treat patients with statin-resistant hypercholesterolemia to reduce the risk of cardiovascular events [5, 6].

In addition to its fundamental role in cholesterol metabolism PCSK9 also regulates host-defense responses. The expression and secretion of PCSK9 are upregulated by inflammatory stimuli via the induction of mitochondrial reactive oxygen species (mtROS) and the NF-κB signaling pathway [7, 8]. The elevated PCSK9 levels are shown to promote pro-inflammatory and inhibit anti-inflammatory cytokines [9]. In septic patients, pathogenic lipids such as lipopolysaccharide (LPS, Gram-negative bacteria), lipoteichoic acid (LTA, Gram-positive bacteria), and phospholipomannan (PLM, fungal organisms) bind to the host's pattern-recognition receptors (PRRs) and ultimately trigger an uncontrolled systemic inflammatory response. These lipids are carried in the circulation within lipoprotein particles and removed through binding to LDLR and VLDLR to become detoxified by the liver [10]. In view of this, the upregulation of LDLR expression in the liver by inhibiting PCSK9 has recently emerged as a potential strategy to reduce exacerbated inflammation in septic patients [11, 12]. According to clinicaltrials.gov, the

first clinical phase II/III trials were started in January 2019 (NCT03634293 and NCT03869073).

Boyd and colleagues have investigated the plasma PCSK9 levels in patients that entered the emergency department with suspected sepsis [13]. The authors found that PCSK9 was markedly increased in the patients compared to reference values from healthy individuals and that there was a positive correlation between high PCSK9 levels and cardiovascular and respiratory failure. Two other studies have explored the circulating PCSK9 levels in critically ill patients that were admitted into intensive care units (ICU) [14, 15]. The results showed that plasma concentrations were similarly elevated in septic and non-septic patients and were higher than normal in ICU patients with severe trauma injury, which implicates that plasma PCSK9 is also upregulated by non-infectious stress to the body.

In this study, we quantitated the plasma PCSK9 levels during the first four days of hospitalization of 481 patients that had blood culture-positive bacteremia when admitted to the emergency department (ED). Specifically, we explored the pathogen and site-of-infection dependent effects on plasma PCSK9 and its correlation with patient characteristics and outcome. Our results demonstrate that a reduced PCSK9 plasma response to an infection is significantly associated with a fatal prognosis.

Materials and methods

Bacteremia patient cohort

Tampere University Hospital is a tertiary hospital with a catchment population of ca. 524 700 inhabitants in the Pirkanmaa County, Finland. All of the blood culture-positive patients that entered the hospital ED between Mar 1, 2012 and Feb 28, 2014 were included in the study cohort (contaminants were excluded). The day 0 EDTA blood sample was obtained within 24 hours after the patient was admitted to the ED. Follow-up plasma samples were collected from patients alongside treatment-based routine laboratory testing. Blood cultures were collected in BacT/Alert Aerobic (FA Plus) and Anaerobic (FN Plus) blood culture bottles and placed in an automated microbial detection system, BacT/Alert 3D (bioMérieux, Marcy l'Etoile, France). The clinical data of the patients were collected retrospectively from patient records. Diagnoses of severe sepsis and septic shock were made according to Sepsis-2 consensus definitions [16]. A criterion for the qSOFA score was calculated based on Sepsis-3 definitions [17]. The Pitt Bacteremia Score was calculated as presented by Korvick et al [18]. The site of infection was determined retrospectively. A more detailed description of the patient cohort is available in our previous publications [19, 20]. In addition, we used 17 plasma samples from a separate patient cohort as healthy controls. These samples were taken one year after surviving from bacteremia [21]. The median PCSK9 level in the control samples was 188 ng/ml (inter-quartile range (IQR) 139-264), which is in line with the result of a meta-analysis describing the level of PCSK9 in healthy individuals [13]. The study was approved by the Ethics Committee of Tampere University Hospital, Finland (permit# R11099). The need for informed consent was waived as no additional blood sampling was needed and routine patient care was not modified.

Plasma PCSK9 level measurement

Plasma PCSK9 was quantitated from plasma samples using a commercially available ELISA assay (R&D Systems, cat#DPC900) and the manufacturer's recommended protocol. To validate the reproducibility of the PCSK9 ELISA, some of the samples were measured as duplicates on the same or separate plates. The intra-array and between-array variations of the samples were on average 3.5% of the defined final concentration.

Statistical analyses

SPSS version 22.0 software (IBM Corp., NY, USA) and R (version 3.4.4. https://www.r-project.org) were used for statistical analyses. Nonparametric data were analyzed using the Mann–Whitney U-test. The Pearson's product-moment coefficient was used to test the correlation. A P-value of <0.05 was considered significant.

Results

PCSK9 levels are equally upregulated in Gram-positive and Gram-negative bacterial infections and show a positive correlation with CRP

During the study period a total of 800 consecutive blood culture-positive cases were admitted to the ED [19]. After excluding the contaminants (n=136) and disregarding missed samples due to technical reasons we collected the day 0 plasma samples from 481 blood-culture-positive cases. As ten patients were admitted twice and one patient three times, the number of individual patients in the cohort is 469. Follow-up plasma samples were collected from patients alongside treatment-based routine laboratory testing within the first four days of hospitalization. The numbers of plasma samples declined during the follow-up because patients were discharged or they died, for example. The final numbers of the plasma samples were: day 0: 481, day 1: 446, day 2: 389, day 3: 300, day 4: 137.

The median level of day 0 plasma PCSK9 in patients (376 ng/ml, IQR 293-483) was significantly higher than in the control group (188 ng/ml, IQR 139-264, $p=0.001$). The PCSK9 level was elevated to the same degree in cases of both a Gram-positive (381 ng/ml, IQR 292-493 $p<0.001$) and a Gram-negative (median level 380 ng/ml, IQR 306-465 ng/ml $p<0.001$) bacterial infection, and it remained at equally high levels throughout the sampling period (Figure 1). We evaluated the correlation between plasma PCSK9 and infection-associated markers. Using Pearson's product-moment analysis we could not observe a significant correlation between PCSK9 plasma levels and body temperature, leukocyte numbers or cell-free DNA (data not shown). In contrast, there was a significant positive correlation between the PCSK9 and CRP levels in plasma samples that were taken during the first 4 days of hospitalization (Figure 2 and supplementary Figure 2, day 0: $r=0.324$, $p<0.001$, day 1: $r=0.270$, $p<0.001$, day 2: $r=0.251$, $p<0.001$, day 3: $r=0.256$, $p<0.001$, day 4: $r=0.214$, $p<0.001$).

The plasma PCSK9 levels are highest in patients with a lower respiratory tract infection and pneumococcal bacteremia

The plasma PCSK9 levels in relation to different causative organisms are shown in Table 1 (day 0) and in supplementary Figure 1 (days 1-4). When the microbes or microbe groups with a low number of cases (less than 5 samples) were excluded, only patients with a *Streptococcus pneumoniae* –infection showed significantly higher levels of PCSK9 compared to other cases on day 0 (476 ng/ml, IQR 319-561, p=0.003). The lowest levels of PCSK9 were seen in patients with polymicrobial bacteremia (311 mg/ml IQR 235-501), but this difference was not statistically significant. When the patients were grouped based on the site of infection, the lowest PCSK9 levels were found in individuals suffering from an unknown or unclassifiable infection (Table 2, 352ng/ml, IQR 262-474, p=0.039). Patients with a lower respiratory tract infection had especially high plasma PCSK9 levels (472 ng/ml, IQR 315-557, p=0.007), which is in accordance with the findings from microbe grouping as *Streptococcus pneumoniae* is the major pneumonia-causing pathogen.

The association of plasma PCSK9 levels with characteristics of bacteremia patients, underlying conditions and severity of the bacteremia

Table 3 shows the plasma PCSK9 levels on day 0 stratified by various patient characteristics, underlying conditions and the severity of the bacteremia. The PCSK9 level was significantly lower in males (p=0.001) and in patients with liver disease (p<0.001) or in patients who used alcohol abusively (p<0.001). In contrast, there was no significant association between PCSK9 and age, cardiovascular disease, diabetes, kidney disease, tumors, hematological malignancies or use of per oral corticosteroids. On the day of admission, 116 cases (24%) were using statins. Simvastatin (63%) and atorvastatin (28%) were the most common with an average dose of 25 mg and 22 mg, respectively. Patients using statins had significantly higher plasma PCSK9

levels (patients not on statins, 363 ng/ml, IQR 283-468, vs. statin users 413 ng/ml, IQR 335-532, p<0.001).

In our patient cohort, we found that critically ill patients needing vasopressor treatment had significantly lower plasma PCSK9 on day 0 (vasopressor treatment: 316 ng/ml, IQR 256-438 vs. no vasopressor treatment 379 ng/ml, IQR 298-486, p=0.026). There was also a nearly significant trend for lower PCSK9 levels in patients that required ICU care (ICU patients 343ng/ml, IQR 256-431 vs. others 379ng/ml, IQR 297-486, p=0.050). The severity of bacteremia indicators qSOFA score ≥2, Pitt Bacteraemia Score ≥4, severe sepsis (according to Sepsis-2 definition) or septic shock (Sepsis-2) were not significantly associated with PCSK9 levels (p=0.564, p=0.323, p=0.120 and p=0.110, respectively).

Lower plasma PCSK9 levels in patients with bacteremia are associated with mortality

In our cohort, the day 7 case fatality rate was 9%. On day 0 the median level of circulating PCSK9 was 306 ng/ml (IQR 247-454) in patients who died by day 7 and 381 ng/ml (IQR 298-486) in patients who survived beyond the first week (p=0.022, Table 4). Patients that succumbed by day 28 had also significantly (ca. 20%) lower PCSK9 plasma levels on days 0, 1, and 2 than survivors. The occurrence of true infection-associated deaths is trumped by underlying disease-based mortality when patients are followed beyond the first month from the diagnosis of bacteremia. Nevertheless, lower PCSK9 levels on day 0 showed also a significant association with the 90-day mortality (death by day 90 320ng/ml, IQR 249-442 vs. survivors 388ng/ml, IQR 307-489, p=0.001).

Finally, we evaluated whether the association between PCSK9 levels and death was dependent on patient characteristics or underlying conditions that affect PCSK9 levels. In males, low PCSK9 levels remained significantly associated with day 7 mortality (p=0.023). The patients without statin medication also showed a significant association between their PCSK9 levels on

day 0 and lethality (death by day 7: 291 ng/ml IQR 214-438 vs. survivors: 373 ng/ml, IQR 292-473 ng/ml, p=0.007). Furthermore, there were 49 cases with liver disease that had a high day 7 and day 28 fatality rate (20% and 31%, respectively) and a low median level of PCSK9 on day 0 (284 ng/ml, IQR 176-352). In patients with a healthy liver, the association between low PCSK9 and death by day 7 or 28 was statistically insignificant (p=0.336 and p=0.059, respectively), indicating that the association between PCSK9 and mortality is partly dependent on liver disease.

Discussion

To our knowledge, this is the first study that evaluated the level of plasma PCSK9 in blood-culture positive infections in a large ED patient cohort. Our results demonstrate that plasma PCSK9 levels are upregulated in bacteremia patients irrespective of the type of the causative organism or infection focus. Especially high PCSK9 levels were seen in patients with a *Streptococcus pneumonia* infection and lower respiratory tract infections. PCSK9 levels show a highly significant positive correlation with plasma CRP, and patients with liver disease released less PCSK9 into the plasma. Importantly, our findings imply that plasma PCSK9 resembles the liver-produced acute phase reactants [22], and a lowered PCSK9 response has a significant association with a fatal prognosis.

PCSK9 levels are elevated by an inflammatory response [23] and in critically ill ICU patients [14, 15], but how PCSK9 levels behave in different types of bacterial infections has not previously been explored. Our bacteremia cases had a median day 0 PCSK9 level of 376 ng/ml, which is ca. double of what was reported in a meta-analysis of 15 PCSK9 cholesterol studies, wherein the final pooled 95% confidence interval was 170-220 ng/ml [13]. Notably, the patients in our small control group, where the bacteremia had been cured, had normal levels of plasma PCSK9, which indicates that the elevation of PCSK9 induced by the infection does not persist in patients. We could not detect any differences in the levels of plasma PCSK9 between patients with Gram-negative or Gram-positive infections. This is in accordance with the findings demonstrating that the major lipoproteins of both Gram-negative (LPS) and Gram-positive (LTA) bacteria are removed from the circulation in a LDLR/PCSK9 dependent manner [14].

Lower respiratory infections, especially those caused by *Streptococcus pneumoniae*, typically result in a severe bodily inflammatory condition as confirmed by a drastic raise in CRP [24]. Similarly, high PCSK9 levels in patients with a lower respiratory tract infection and

Streptococcus pneumoniae growth in blood culture imply that PCSK9 levels are associated with the strength of the inflammatory response. Indeed, when we evaluated the associations between infection markers, PCSK9 showed highly significant correlations with CRP levels on days 0-4 after admission to the ED. This is in line with a previous study where PCSK9 and TNFα plasma levels were reported to show a positive correlation in healthy adults [9]. Intriguingly, a recent study found that PCSK9 levels were lowered in patients after elective abdominal aortic aneurysm repair surgery [25], which indicates that PCSK9 is not universally upregulated upon bodily stress. In our study, the majority of patients (445/481) started receiving antibiotic treatment on day 0, which could in theory increase the amount of pathogenic lipids in circulation. However, we did not observe any significant changes in the PCSK9 levels during the first four days hospitalization.

In addition to inflammatory conditions, plasma PCSK9 levels are elevated by the use of lipid-lowering drugs, such as statins [26]. We observed a similar upregulation of plasma PCSK9 in bacteremia patients using statins, but also identified underlying conditions that were associated with lower plasma PCSK9 levels (male sex, liver disease and abusive alcohol use). The PCSK9 protein is primarily produced by liver cells, and low serum PCSK9 levels negatively correlate with poor liver function in non-infectious patients [27]. However, PCSK9 is also expressed in other organs, including the brain and small intestine [28, 29]. Our findings showing the dependency between liver disease and PCSK9 indicate that in bacteremia patients the main source for circulating PCSK9 is the liver, rather than any other organ.

We found that the reduced elevation of PCSK9 in the plasma of bacteremia patients is significantly associated with day 7, 28 and 90 mortalities. Higher circulating PCSK9 levels in patients with suspected sepsis have previously been shown to be associated with the development of acute organ failure, indicating that higher PCSK9 levels are found in more critically ill patients [13]. Our results are conflicting as patients needing mechanical ventilation

or vasopressors had significantly lower levels of PCSK9 than those who did not require intensive care. Most importantly, patients that were deceased by day 7, 28 or 90 also had statistically significantly reduced levels of plasma PCSK9. Notably, the 28 day mortality was higher in our cohort than in the previous study by Boyd and colleagues [13] (15% vs. 5%), which could reflect the fact that we only included culture-positive cases.

The exclusion of statin users or restricting the analyses to males did not abolish the association between a lowered PCSK9 response and death. In contrast, the lethality was at least partly dependent on the liver disease as well as affiliated abusive alcohol usage. This is in line with the previous findings with non-infectious patients with end-stage liver disease [27]. Schengel and colleagues found that liver patients had lower serum PCSK9 levels (medium 106.39ng/ml) than healthy populations, and that there was a negative correlation between serum PCSK9 and markers of liver function. In our bacteremia cohort, PCSK9 levels are upregulated by infection also in patients with liver disease, but by a lesser magnitude. It is thus important to note that even higher-than-normal plasma PCSK9 levels can be associated with lethality in bacteremia patients.

PCSK9 blocking antibodies have been proposed to be beneficial in reducing the lipoprotein-triggered inflammation in patients with a systemic infectious disease [11, 12]. Our results show that higher plasma PCSK9 levels are not detrimental to bacteremia patients. It is also important to note that in an experimental model the inhibition of PCSK9 was not found to improve lipopolysaccharide-induced mortality in mice [30], and that a slight rise in the incidence of upper respiratory tract infections was observed in patients using PCSK9 inhibitors (2.1% alirocumab vs. 1.1% placebo) [31, 32]. Moreover, a recent study showed that a PCSK9 loss-of-function variant was not associated with an increased risk of hospitalization for a serious infection [33]. Hence, the use of PCSK9 inhibitors in sepsis remains controversial.

Our study has some limitations that need to be addressed. This was a single-centered tertiary hospital study involving only culture-positive patients. Our results should not be extrapolated to culture-negative cases with an infection and should be reproduced using an independent study cohort. A few cases of culture-positive result were missed due to technical problems in sample collection/storage, but the number of these samples is low and the possible bias has been analyzed to be insignificant [19].

In conclusion, PCSK9 remains in the limelight when novel diagnostic and treatment options for systemic infectious diseases are considered. As the PCSK9 inhibitors have only recently been added to the clinical toolbox, our understanding of their effects in the course of severe infections is still sparse. As data from the PCSK9 plasma level measurements accumulate, we will begin to better understand PCSK9s significance in health and disease.

Potential conflicts of interest:

Juha Rannikko: No conflict

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Table 1. Causative organisms and the median level of PCSK9 on the day of admission to the emergency department.

Organisms	n (%)	Median plasma PCSK9 level (ng/ml) with inter-quartile range
Gram-positive	**213 (44)**	**381 (292-493)[1]**
Staphylococcus aureus	71 (15)	358 (293-479)
Coagulase-negative Staph.	11 (2)	421 (278-484)
Streptococcus pneumoniae	46 (10)	476 (319-561)
β-hemolytic streptococci	43 (9)	382 (288-498)
Viridans streptococci	21 (4)	353 (287-470)
Enterococci	17 (4)	333 (267-414)
Other Gram-positive	4 (1)	618 (327-958)
Gram-negative	**222 (46)**	**380 (306-465)[2]**
E. coli	156 (32)	388 (306-442)
Klebsiella sp.	23 (5)	392 (340-520)
Pseudomonas aeruginosa	18 (4)	345 (292-416)
Other Gram-negative	25 (5)	315 (226-494)
Others	**46 (10)**	**339 (261-488)[3]**
Anaerobes	15 (3)	374 (318-464)
Fungi	1 (0.2)	275 (275-275)
Polymicrobial	30 (6)	311 (235-501)
All	**481 (100)**	**376 (293-483)**

[1] Day 7 case fatality rate 5.6%
[2] Day 7 case fatality rate 9.5%
[3] Day 7 case fatality rate 23.9%

Table 2. Site of infection, number of deceased patients by day 7 and the median level of PCSK9 (ng/ml) on the day of admission to the emergency department. There were 29 cases with two different infection sites.

Site of infection	n (%)	Deceased n (%)	Median plasma PCSK9 level (ng/ml) with inter-quartile range
Unknown/unclassifiable	116 (24)	18 (16)	352 (262-474)
Urinary tract infection	134 (28)	9 (7)	388 (303-468)
Intra-abdominal	83 (17)	12 (15)	365 (291-443)
Skin, soft tissue and bones	65 (14)	2 (3)	377 (306-527)
Lower respiratory tract	48 (10)	4 (8)	472 (315-557)
Surgical site or foreign body	36 (7)	0 (0)	367 (287-474)
Others	19 (4)	1 (5)	374 (310-447)

Table 3. Plasma PCSK9 levels on the day of admission to the emergency department stratified by various characteristics, underlying conditions and the severity of the bacteremia.

		Plasma PCSK9 level(ng/ml) on day of admission		
		factor present, median (quartiles)	**factor absent, median (quartiles)**	**p-value**
Characteristics and underlying conditions	**n (%)**			
Male	253 (53)	356 (273-472)	405 (310-492)	0.001
Age over 60 years	333 (69)	381 (298-486)	368 (275-478)	0.223
Age over 80 years	107 (22)	379 (304-477)	376 (289-485)	0.804
Cardiovascular disease	155 (32)	388 (299-479)	371 (291-484)	0.868
Diabetes any type	137 (29)	375 (295-468)	379 (292-487)	0.591
Chronic kidney disease[4]	66 (14)	364 (297-440)	379 (292-487)	0.152
Liver disease	49 (10)	284 (176-352)	389 (305-488)	<0.001
Alcohol abuse[5]	49 (10)	264 (190-388)	388 (306-489)	<0.001
Solid tumour with metastasis	55 (11)	399 (291-484)	375 (293-482)	0.783
Haematological malignancy	45 (9)	382 (308-485)	376 (291-484)	0.508
Use of oral corticosteroids	92 (19)	415 (306-487)	370 (291-480)	0.125
Use of statins	116 (24)	413 (335-532)	363 (283-468)	<0.001
Severity of bacteremia	**n (%)**			
qSOFA score ≥2[6]	128 (28)	378 (263-489)	376 (299-480)	0.564
Need of vasopressor	49 (10)	316 (256-438)	379 (298-486)	0.026
Need of mechanical ventilation	24 (5)	309 (235-593)	377 (395-481)	0.631
Severe sepsis[7]	145 (30)	363 (266-477)	380 (301-486)	0.120
Septic shock[4]	36 (8)	347 (256-438)	378 (296-486)	0.110
Admitted from ED to ICU	44 (9)	343 (256-431)	379 (297-486)	0.050

[4] History of creatinine more than 120 µmol/l
[5] Social or medical problems of alcohol abuse in the past 12 months
[6] Data available on 458 cases
[7] According to Sepsis-2 definition

Pitt Bacteraemia Score ≥4[8]	58 (12)	344 (262-493)	380 (298-482)	0.323
Death by day 7	45 (9)	306 (257-454)	381 (298-486)	0.022
Death by day 28	71 (15)	308 (242-431)	387 (299-487)	0.001
Death by day 90	101 (21)	320 (249-441)	388 (307-489)	0.001

[8] Data available on 474 cases

Table 4. Plasma PCSK9 levels during days 0 to 4 after admission to the emergency department in all cases and in deceased individuals and survivors by days 7 and 28.

Days after admission	Plasma PCSK9 (ng/ml), median (quartiles)						
	All	Deceased by day 7	Survivors by day 7	p-value	Deceased by day 28	Survivors by day 28	p-value
Day 0	376 (293-483)	306 (247-454)	381 (298-486)	0.022	308 (242-431)	387 (299-487)	0.001
Day 1	383 (296-484)	351 (228-435)	385 (300-485)	0.062	348 (213-429)	389 (303-488)	0.003
Day 2	381 (295-478)	321 (248-429)	385 (298-482)	0.030	330 (219-446)	387 (300-490)	0.005
Day 3	360 (283-465)	348 (225-428)	364 (284-472)	0.216	355 (268-438)	360 (284-470)	0.717
Day 4	356 (274-452)	324 (189-440)	359 (274-457)	0.557	344 (242-444)	356 (275-460)	0.646
Maximum level day 0-4	455 (362-566)	360 (256-502)	457 (374-559)	<0.001	378 (256-506)	470 (376-560)	<0.001

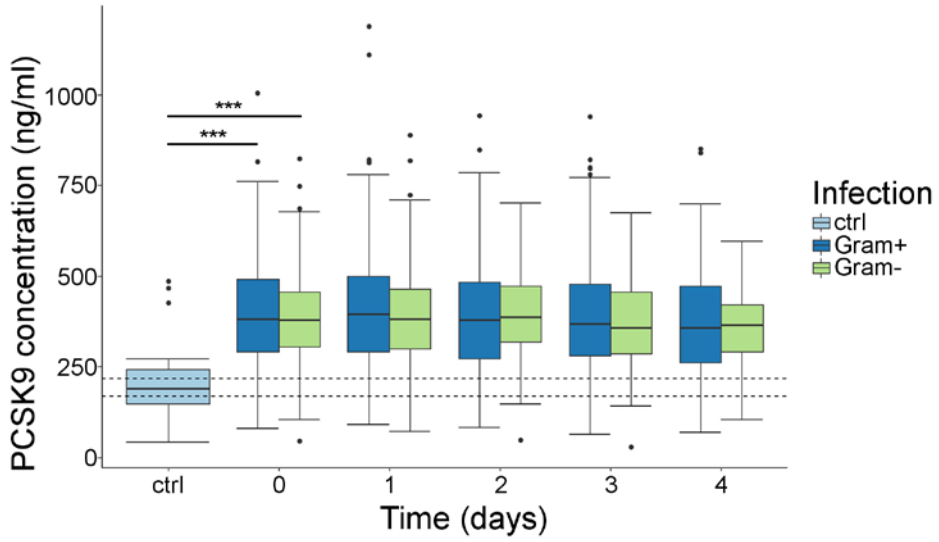

Figure 1. PCSK9 plasma levels of bacteremia patients

PCSK9 concentration of 481 bacteremia patients and 17 controls were measured using ELISA. Box plot representation of the PCSK9 plasma levels in patients with a Gram-positive or Gram-negative infection and in controls on days 0 – 4 are shown. The box represents the interquartile range (25^{th}–75^{th} percentiles) and the whiskers represent the 10^{th}–90^{th} percentiles. Dashed lines mark the reported normal plasma PCSK9 range (170-220ng/ml) [13]. PCSK9 levels of Gram-positive and Gram-negative groups were compared to a control group (ctrl) and both were significantly elevated (*** $p<0.001$, Welch's two sample t tests).

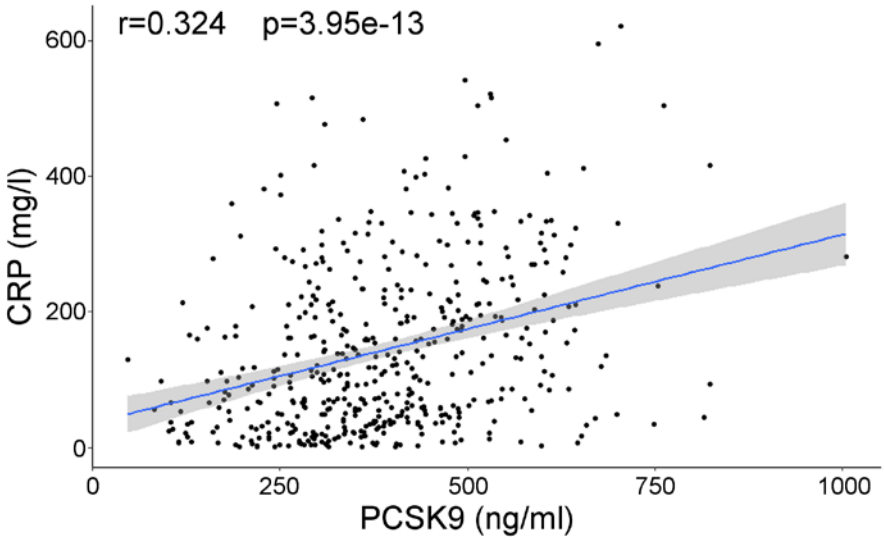

Figure 2. Correlation between plasma PCSK9 and CRP levels in bacteremia patients

PCSK9 and CRP levels were measured from bacteremia patients on the day of admission to the emergency department. Plasma PCSK9 levels are plotted against CRP and the correlation was studied by calculating the Pearson's product-moment correlation coefficient (r). The shadowed area shows the 95% confidence interval.