The Role of PCSK9 in Infectious Diseases

Laura Magnasco[1], Chiara Sepulcri[1,2], Roberta Maria Antonello[3], Stefano Di Bella[4], Laura Labate[1,2], Roberto Luzzati[4], Daniele Roberto Giacobbe[1,2,*] and Matteo Bassetti[1,2]

[1]Infectious Diseases Unit, San Martino Policlinico Hospital - IRCCS, Genoa, Italy; [2]Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy; [3]Department of Infectious Diseases, University of Trieste, Trieste, Italy; [4]Clinical Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

Abstract: ***Background*:** In recent years, many aspects of the physiological role of PCSK9 have been elucidated, in particular regarding its role in lipid metabolism, cardiovascular risk but also its role in innate immunity. Increasing evidence is available on the involvement of PCSK9 in the pathogenesis of viral infections, mainly HCV, as well as in the regulation of host response to bacterial infections, mainly sepsis and septic shock. Moreover, the action of PCSK9 has been investigated as a crucial step in the pathogenesis of malaria infection and disease severity.

***Objective*:** Aim of this paper is to review available published literature on the role of PCSK9 in a wide array of infectious diseases.

***Conclusion*:** Besides the ongoing investigation on PCSK9 inhibition among HIV-infected patients for the treatment of HIV- and ART-related hyperlipidemia, preclinical studies indicate how PCSK9 is involved in reducing the replication of HCV. Moreover, a protective role of PCSK9 inhibition has also been proposed against dengue and SARS-CoV-2 viral infections. Interestingly, high plasmatic PCSK9 levels have been described in patients with sepsis. Finally, a loss of function in the PCSK9-encoding gene has been reported to possibly reduce mortality in malaria infection.

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1. INTRODUCTION

The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a Ca^{2+}-dependent serine protease belonging to the proprotein convertase family and consisting of a 692 amino acid sequence. Its structure is divided into an N-terminal prodomain, a subtilisin-like catalytic domain and a C-terminal domain. The catalytic domain is responsible for mediating the self-assemblage of the convertase, while the C-terminal domain is pivotal in binding the hepatic low-density lipoproteins receptor (LDLR). Indeed, it is nowadays well-known that PCSK9 physiologically enhances the endosomal and lysosomal degradation of hepatic LDLR, leading to an increased level of circulating LDL cholesterol [1]. Because of this mechanism of action, this molecule has been extensively investigated in recent years as a therapeutic target in the management of hyperlipidemia [2]. Monoclonal antibodies targeting epitopes in the catalytic domain of PCSK9, next to the binding site with LDLR, sterically prevent the binding of PCSK9 to their target [3]. Two of these antibodies have been FDA-approved for clinical use: evolocumab and alirocumab. Evolocumab is a 142 kDa human IgG2, while alirocumab is a fully human IgG1 monoclonal antibody [4]. Both these drugs are administered subcutaneously, and their rather long half-life (ranging from 11 to 20 days) allows for administration every 2 weeks [5]. To date, the use of PCSK9 inhibitors has been recommended by international guidelines to reduce cardiovascular events

*Address correspondence to this author at the Infectious Diseases Unit, San Martino Policlinico Hospital - IRCCS, Genoa, Italy;
Tel: +393463979501; Fax: +390105556606;
E-mail: danieleroberto.giacobbe@hsanmartino.it

among high risk patients [5]. However, LDLR and other lipoproteins also play a role in the pathogenesis of viral infections, in particular in the entry of hepatitis C virus (HCV) in human hepatic cells [6, 7], as well as the in the regulation of the host response to bacterial infections, mainly sepsis and septic shock [8-13]. Moreover, a growing body of evidence is becoming available with regards to the role of lipid metabolism in the setting of malaria infection [14, 15]. Much interest is arising in the use of PCSK9 inhibitors also in the setting of treatment of infections themselves or of the immune dysregulation that derives from such infections. Indeed, many clinical trials are ongoing to evaluate the role of evolocumab and alirocumab in this light.

The aim of this review is to summarize currently available evidence about the role of PCSK9 in the pathogenesis and treatment of infectious diseases.

2. MATERIALS AND METHODS

A literature search was performed in the Pubmed online database. The keywords used were "PCSK9 AND infection", and results published up to November 26th, 2020 were reviewed. This search retrieved 143 results. The authors performed a first title screening to check for consistency with the topic treated in this review, and non-consistent papers were excluded. Therefore, 82 articles were left for full-text screening. References of the included papers were screened to check for possible further missed evidence regarding the topic.

3. MINI-REVIEW

3.1. The Role of PCSK9 in Inflammation and Immunity

Since its discovery in 2003, PCSK9 has progressively gained the attention of the scientific community and international research groups because of its decisive role in lipid metabolism and potential role in inflammatory pathways, namely atherogenesis, but also infection. It is now well established that inhibiting PCSK9 leads to a reduction in circulating LDL cholesterol (LDL-C) and reduces cardiovascular events as it was demonstrated in the FOURIER trial [16]. Furthermore, PCSK9 is directly associated with the amount of necrotic core tissue in coronary atherosclerotic plaque independently from LDL-C levels and from statin use, as demonstrated in the ATHEROREMO-IVUS study [17], which included 581 patients undergoing coronary angiography for either unstable angina or acute coronary syndrome. The levels of PCSK9 are also correlated to infarct size area in a PCSK9 knock-out murine model, as reported in a study by Ding *et al.* [18]. This data suggest a role of PCSK9 in atherogenesis that goes beyond its direct relation to LDL metabolism and can be attributed to its involvement in inflammatory pathways [19]. Indeed, PCSK9 is in close relationship with inflammatory cytokines, increasing the expression of some of them, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and C-X-C motif chemokine ligand 2 (CXCL2) *via* the toll-like receptor 4/nuclear factor-κB (TLR4/NF-κB)-dependent pathway in the atherosclerotic plaque [20, 21]. In turn, PCSK9 expression in macrophages and vascular smooth muscle cells (VSMCs) is induced by a variety of stimuli, among which are oxidized LDLs, bacterial lipopolysaccharide (LPS) and TNF-α, which promote apoptosis of VSMCs *via* the mammalian target of rapamycin (mTOR) pathway. Moreover, PCSK9 interacts in a positive feedback fashion with the lectin-like oxidised-LDL receptor-1 (LOX-1), the scavenger receptor for oxidized LDLs and an important player in atherosclerotic plaque formation, especially after an inflammatory stimulus [22, 23]. Besides VCSMs and the liver, the main site of PCSK9 expression, this protein is also expressed in lungs and intestinal tissue, in areas that are in close contact with the endogenous microbiome. In these sites, PCSK9 is expressed by activated macrophages, following an inflammatory stimulus (such as LPS) and it plays an important role in the innate immune response [24]. During bacteremia, LPS and, to some extent, lipoteichoic acid (LTA – constituent of the Gram-positive bacteria wall) enters the hepatocyte through LDLR, which is the main target of PCSK9 activity [10, 25]. By downregulating LDLR expression, PCSK9 reduces LPS uptake and clearance by hepatocytes, thus leading to an increased host inflammatory response, both as a result of increased endotoxin circulation and the pro-inflammatory activity of PCSK9 *per se*. This enhanced pro-inflammatory state is observed in sepsis, and in particular in its most extreme manifestation, that is septic shock. This effect was demonstrated *in vivo* in murine models [13] and in humans by Walley *et al.* [11], who rigorously demonstrated a reduction in plasmatic levels of TNF-α and IL-6 in patients carrying a loss-of-function (LOF) mutation in PCSK9 both in a cohort of patients affected by septic shock and in a cohort of healthy volunteers undertaking an LPS intravenous infusion. These findings explained the mechanisms underlying the phenomenon by which reduced levels of LDL and high-density lipoproteins (HDL) correlates with poor outcome and greater disease severity in sepsis [26] and led to trials involving the use of PCSK9 inhibitors in sepsis, which failed to demonstrate a beneficial effect on mortality in

murine models [27, 28]. The possible explanation of poor efficacy of PCSK9 inhibitors in sepsis and septic shock could be attributed to the role of PCSK9 in the regulation of cholesterol efflux: by degrading LDLR, PCSK9 inhibits reverse cholesterol transport to the liver from the periphery, leading to an increased amount of cholesterol, free to enter macrophages and thus exerting an anti-toxin function [9]. The conflicting role of PCSK9 in inflammation is also highlighted in the case of rheumatoid arthritis, where PCSK9 levels were found to be lower in affected patients compared to matched healthy controls [29], configuring a so-called "paradox" in the relation between lipid metabolism, PCSK9 and inflammation [30]. In addition, the anti-inflammatory and immune-modulating properties of PCSK9 have also been explored in cancer: in a murine model of breast cancer, it was demonstrated that the nanoliposomal anti-PCSK9 vaccine was effective in reducing PCSK9 levels and could moderately improve survival [31]. In a recently published study [32], a potential effect of PCSK9 inhibitors in association to anti-programmed death antibodies in immunotherapy was demonstrated *in vivo* in a murine model of cancer, through the regulation of major compatibility complex I expression and thus influencing cellular immunity, namely tumor infiltration by T lymphocytes, which is at the basis of immune-therapy.

A deeper understanding of the role of PCSK9 in regulating the immune response might give way to possible therapeutic implications targeting this enzyme.

3.2. The Role of PCSK9 in HIV Infection

The advent of highly effective antiretroviral treatment (ART) for human immunodeficiency virus (HIV) infection has drastically changed the life of people living with HIV (PLWHIV), their care-takers and HIV specialists. Currently, the life expectancy of virologically suppressed patients on effective ART approaches is less than that of non-infected adults [33]. Nevertheless, PLWHIV experience more and earlier comorbidities with respect to non-infected individuals, highlighting the importance of medical follow-up and a holistic medical approach beyond the infection *per se*. One of the main comorbidities PLWHIV face is cardiovascular disease, for which PLWHIV are at increased risk due to multiple factors, including higher prevalence of traditional risk factors (*e.g.*, tobacco use, not healthy lifestyle) but also specific HIV-related factors. Indeed, the virus itself is demonstrated to impair cholesterol efflux from infected and non-infected cells through its action on the Nef protein, which inhibits the activity of ATP binding cassette transporter A1 [34]. This action results in lower levels of total cholesterol, LDL-C and HDL-C, together with higher levels of small-dense LDLs and triglycerides, thus configuring an atherogenic phenotype [35, 36]. The pathogenesis of these changes has not been fully elucidated and could be the result of the action of pro-inflammatory cytokines, such as TNF-α, or more broadly of the state of systemic immune-activation experienced by PLWHIV [37]. On the other hand, also ART plays an important role in increasing the cardiovascular risk among PLWHIV: protease inhibitors (boosted with ritonavir or cobicistat) have long been known to cause dysregulation of lipid metabolism leading to hypertriglyceridemia, decrease in HDL-C and increase in total and LDL cholesterol [38]. In recent years, also other classes of compounds that are being increasingly used in modern ART, such as integrase strand transfer inhibitors or tenofovir alafenamide, have been associated with lipid metabolism dysregulation [39, 40]. Besides, co-infection with HCV has been demonstrated to further increase the risk of atherosclerosis [41]. This evidence altogether led to include HIV infection among the risk-enhancing factors for atherosclerotic cardiovascular disease in the US and European guidelines for cardiovascular disease [42, 43], and prompted the need for intensive cholesterol lowering treatment in this particular population. To date, statins are the mainstay of cholesterol lowering treatment but their use is often limited among PLWHIV because of comorbidities (*i.e.*, renal impairment, liver dysfunction) and drug-drug interaction with ART regimens. Given these challenges, PCSK9 and PCSK9 inhibitors were welcomed by HIV specialists as possible tools to control dyslipidemia in this setting. PCSK9 levels in HIV patients have been first assessed in a multicenter, retrospective study by Kohli *et al.* [44], in which the plasmatic levels of PCSK9 were evaluated in 385 HIV mono-infected patients, 110 patients co-infected with HCV and 72 control patients with no viral infection. No increase in PCSK9 among HIV mono-infected patients was noted when compared to non-infected controls, while increased PCSK9 levels were found in co-infected patients, with respect to both control and mono-infected ones. Furthermore, the authors coined the term "PCSK9-lipid paradox" to describe the finding that PCSK9 levels were increased in spite of lower LDL-C levels in co-infected patients when compared to mono-infected patients and control, as was known to happen with statin therapy. These findings might be explained by the pro-inflammatory state driven by a viral infection and by hepatic dysmetabolism caused by HCV infection. These results were not completely confirmed in another multicenter but

prospective, cross-sectional study [45], in which the authors again measured PCSK9 plasma levels in 149 PLWHIV (with or without HCV co-infection) compared to 69 non-HIV-infected controls. They concluded that both HIV and HCV alone were associated with an increase in levels of PCSK9, independently from statin use, when compared to uninfected individuals, but that co-infection did not play an additive effect on PCSK9 levels. They also reported a correlation among PLWHIV between PCSK9 levels and systemic monocyte activation, highlighting the role of PCSK9 in inflammation. However, they further investigated the clinical implication of such findings, reporting how PCSK9 levels did not relate with the presence of subclinical atherosclerotic coronary plaques and only modestly to plasmatic LDL-C levels. The different outcomes of the two studies can be partially explained by the study population: in the study by Kohli *et al.* [44], PLWHIV were part of a greater cohort and included both virologically suppressed and non-suppressed patients, while in the second study [45] 99% of the cohort were virologically suppressed. Another difference was in the use of statins in the various groups. Another study that investigated the relation between PCSK9, LDL-C and endothelial function in PLWHIV was performed by Leucker *et al.* [46]. They included a population of 48 PLWHIV under effective ART without coronary artery disease and 15 HIV seronegative subjects, matched to the study population by age and LDL-C levels, in whom they assessed endothelial function by magnetic resonance imaging and measured PCSK9 and LDL-C plasmatic levels. Their results showed how PLWHIV had greater endothelial dysfunction in terms of percentage change of coronary artery cross-sectional area and increased level of PCSK9, independently from statin use. PCSK9 levels were inversely associated with coronary endothelial function, and this was considered independent from LDL-C levels given the population pairing. All the above-mentioned studies concluded that PCSK9 inhibition could be a useful tool to treat dyslipidemia and prevent cardiovascular events in PLWHIV.

The first study on the use of PCSK9 inhibitor evolocumab in PLWHIV was conducted by Boccara *et al.* and published this year [47]. It was a multinational, randomized, double-blind study that assessed the efficacy in terms of LDL-C levels decrease and safety of evolocumab *vs.* placebo in 467 PLWHIV, of which the majority had a good immune-virological status. Both the primary and secondary endpoints of the study (percent change in LDL-C baseline to week 24 and achievement of LDL-C < 70 mg/dL and percent change in other plasma lipid and lipoprotein levels, respectively) were met without safety issues. At the time of writing, new studies on lipid lowering strategies in PLWHIV are ongoing, namely, the EPIC-HIV study (NCT03207945) [48] that is assessing the effect of another PCSK9 inhibitor (alirocumab) on arterial inflammation and endothelial function and the EVOLVE study (NCT03500302) [49] that aims at evaluating the role of evolocumab *vs.* placebo in reversing endothelial impairment.

3.3. The Role of PCSK9 in HCV Infection

Almost 2 million new HCV infections are estimated each year, and, as of 2015, 71 million people were living with chronic HCV infection worldwide [50]. Chronic HCV infection is a well-known cause of steatosis, cirrhosis and the most common cause of hepatocellular carcinoma (HCC) [51, 52].

HCV lifecycle is closely tied to lipid metabolism. Indeed, HCV uptake is dependent on the interaction of viral envelope glycoproteins E1 and E2 with both viral and lipid specific receptors (*i.e.*, LDLR, scavenger receptor class B type 1, and Niemann-Pick C1-Like Intracellular Cholesterol Transporter 1) [53]. Moreover, viral assembly is associated with lipid droplets, and it is dependent on the presence of apolipoproteins [54]. It was also observed that the viral particle has a maturation pathway similar to that of very-low-density lipoproteins (VLDL) [54, 55] and that HCV virions in the serum of infected patients are associated with VLDL-like vesicles (known as lipoviroparticles) [56-58]. Alternatively, HCV is shown to affect cholesterol homeostasis, which is restored after antiviral treatment [59]. As far as PCSK9 is concerned in this setting, PCSK9 has proved *in vitro* to negatively regulate 4 hepatocyte surface proteins involved in HCV entry (LDLR, scavenger receptor class B type 1, VLDL receptor and cluster of differentiation 81) [7, 60-64]. Although the mechanisms of action are yet to be elucidated, *in vitro* evidence suggests that PCSK9 can down-regulate HCV replication in a dose-dependent manner [65], while at the same time HCV infection can upregulate the expression of PCSK9 [66], suggesting a complex interplay among the enzyme and the virus. These preclinical observations were confirmed in a prospective, multicenter cohort of 178 patients with chronic liver disease with or without HCC [67]. In this study, serum samples from included patients were analyzed to determine whether a different distribution existed in levels of PSK9 and serum lipids in different sub-groups of patients. Indeed, patients with HCV infection were found to have higher serum levels of PCSK9, inde-

pendently from the presence of HCC, and in a manner directly correlated to HCV RNA titers. Interestingly, the authors underlined how HCV genotype 2 turned out to be the most potent PCSK9-inducer, and it is noteworthy how sustained virological response (SVR) rates after antiviral treatment are higher among patients infected with HCV genotype 2 than with other genotypes (74% *vs.* 68% in genotype 2 and 3, respectively) [68]. This finding supported the observation of another retrospective, monocenter study [69] in which samples from 94 patients with chronic hepatitis C treated with pegylated interferon and ribavirin, with or without the addition of a direct-acting antiviral, were analyzed. Higher PCSK9 plasma levels were noted among those patients in the cohort who achieved SVR.

A prospective, monocenter study enrolling 39 patients in Japan investigated the effects of HCV treatment on lipid metabolism [70]: serum levels of LDL, PCSK9 and microRNA-122 (miR122) were evaluated at different timepoints among patients with chronic HCV genotype 1b infection receiving a 24-week treatment with daclatasvir/asunaprevir. Over the treatment period, serum LDL levels increased, while no significant changes were observed in HDL and triglyceride levels, with a consequent increase in the LDL/HDL ratio. MiR122, a key component of both HCV replication and cholesterol metabolism in hepatocytes [71, 72], did not undergo significant changes. In this study, PCSK9 was quantitatively evaluated both in its active (PCSK9-A) and inactive (PCSK9-I) form: PCSK9-A levels were low at week 4 but constantly increased afterwards up to week 52; on the contrary, PCSK9-I levels steadily decreased over time. This could suggest that, while gradually achieving SVR, PCSK9-A levels increase thanks to a progressive activation of the inactive form.

The complex interaction between lipid metabolism and HCV infection suggests possible therapeutic implications. Indeed, a possible pro-viral effect of statins was postulated, due to their effect of increasing LDLR and Niemann-Pick C1-Like Intracellular Cholesterol Transporter 1, although this hypothesis has been suggested in an *in vitro* study [73], it has not been confirmed in two clinical trials [74, 75].

In addition to PCSK9, other similar proprotein convertases, such as subtilisin/hexin-isoenzyme-1/site-1 (SKI-1/S1P) [76], have been investigated in the setting of HCV infection. *In vitro* evidence showed that when a SKI-1/S1P inhibitor was added to culture cells, HCV replication and infectious particle production were decreased [77]. In a preclinical study investigating a SKI-1/S1P inhibitor (PF-429242) [78], an antiviral effect during the early phases of HCV lifecycle was observed, but further investigations are needed to evaluate the effect of such molecules *in vivo*.

3.4. The Role of PCSK9 in Other Viral Infections

Besides HIV and HCV, the role of PSCK9 has also been investigated in other viral infections. Especially for those infections that are lacking a definite, effective antiviral treatment, deeper understanding of relations occurring among viruses and host factors able to influence the course of diseases, might be particularly relevant in order to develop possible treatments targeting specific pathway molecules. In a recent preclinical study [79], the authors assessed the influence of dengue virus (DENV) infection on PCSK9, and observed that DENV infection induced PCSK9 expression in hypoxemic HuH7 liver-derived cells *in vitro*, thus causing a downregulation of LDLR expression on the cell membrane of infected cells and a subsequent reduction in circulating LDL-C concentrations with a sequester of cholesterol in the intracellular space, responsible for a more favorable proviral medium. Indeed, cholesterol was already known to play a role in DENV infection since DENV infected cells stimulate the production of intracellular cholesterol synthesis, which in turn aids in the formation of the replication complex suitable for DENV [80, 81], and plasma LDL-C levels were found to be negatively correlated to the severity of DENV infection [82]. In light of these findings, cholesterol-lowering treatment with statins had been investigated as a possible therapy for patients infected with DENV, but no efficacy could be demonstrated in a randomized clinical trial [83]. Furthermore, the role of other possible therapeutic targets in lipid metabolism, such as PCSK9, were investigated. An increase in PCSK9 levels was found to be related to increase in DENV titers in human-myeloid derived cells under hypoxemic condition, a surrogate of the lymph nodal microenvironment where DENV replicates in humans. In this study, the authors demonstrated *in vitro* that by inhibiting PCSK9 with a tailored monoclonal antibody (alirocumab), LDLR membrane levels were restored on the infected cell membrane and reduced DENV plaque titers were observed at 24- and 48-hours post-infection. This finding was replicated for all DENV serotypes. Altogether, these findings open up to the possible use of PCSK9 inhibitors in the context of DENV infection, although further clinical studies are paramount.

In addition to flavivirus, also coronaviruses, likewise other RNA viruses (such as porcine reproductive and respiratory syndrome virus) [84], have been found

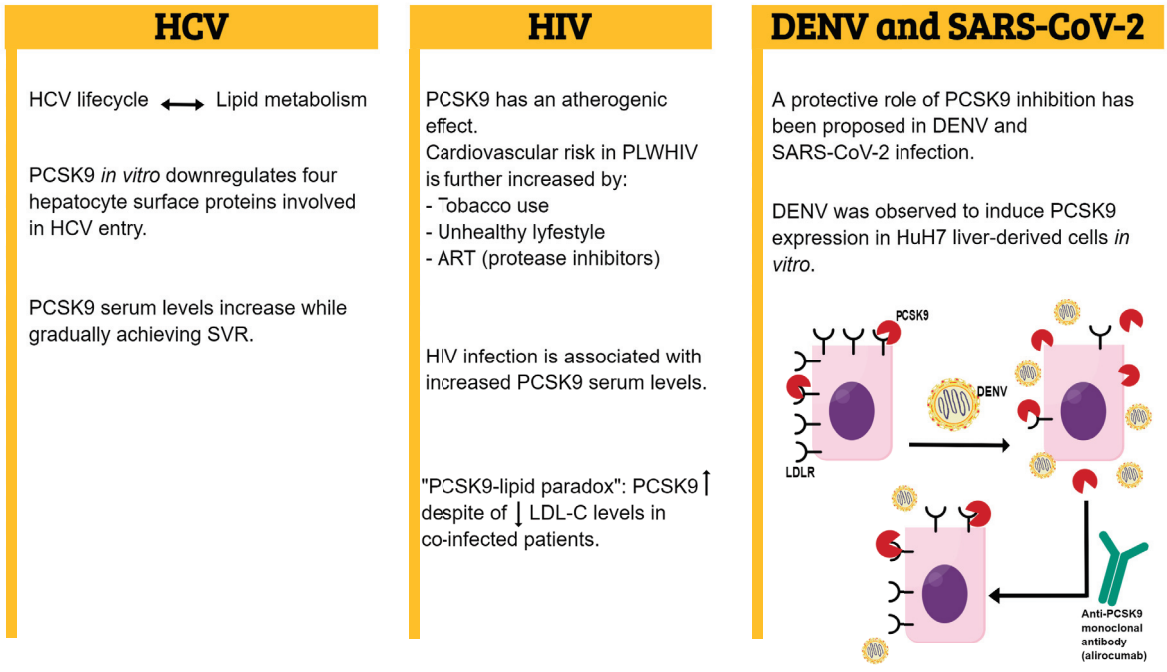

Fig. (1). Role of PCSK9 in viral infections. PCSK9: proprotein convertase subtilisin/kexin type 9; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; PLWHIV: people living with HIV; ART: antiretroviral therapy; DENV: Dengue Virus; SARS-CoV-2: Severe acute respiratory syndrome Coronavirus 2; SVR: sustained virological response; LDL-C: low density lipoprotein cholesterol; LDLR: low density lipoprotein receptor. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

to be profoundly influenced by cholesterol metabolism [85, 86]. This finding acquired new importance with the emergence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic, especially when considering the role that obesity and cardiovascular disease play in enhancing the risk for severe disease. With regards to SARS-CoV-2 infection, a possible role of lipid metabolism in the infection course has been postulated based on evidence from previous similar viral infections, even if a definite demonstration is lacking [87, 88]. Similarly, in the same papers, a benefit on the clinical course of SARS-CoV-2 infection is suggested among patients with familial hypercholesterolemia treated with PCSK9 inhibitors, with or without statins. However, to date, no clinical trial or study on the influence of PCSK9 inhibitors on SARS-CoV-2 infected patients has been published. Fig. (**1**) sums up the role of PCSK9 in viral infections.

3.5. The Role of PCSK9 in Bacterial Sepsis

Sepsis derives from a multifaceted host response to an infecting pathogen that may be significantly amplified by endogenous factors, resulting from early activation of both pro- and anti-inflammatory responses with a sharp imbalance between the two. To date, no effective treatment for the dysregulation of immune response occurring in sepsis and septic shock is available [89]. Many pathogenic lipids contained in microbial cell walls such as LPS in Gram-negative bacteria, LTA in Gram-positive bacteria, and phospholipomannan in fungi [90, 91] are major ligands for TLRs, which prominently mediate the septic inflammatory response in sepsis or septic shock. These pathogenic lipids are initially incorporated into HDLs and subsequently can be transferred to LDLs or VLDLs [92], which are cleared from the blood by the liver, thus reducing the inflammatory response induced by the pathogenic lipids themselves [93]. This uptake in the liver is mediated mostly by LDLR/PCSK9 interaction [25], thus arising interest in the role of PCSK9 in the cytokine storm typical of severe sepsis and its pharmacological inhibition as a possible way to mitigate it [94, 95].

In a randomized controlled trial conducted in a murine model, Walley *et al.* administered intraperitoneally LPS to a group of PCSK9-knockout mice and a control group of healthy mice. They demonstrated a beneficial effect of PCSK9 LOF mutation on the inhibition of inflammatory response (measured as plasma levels of TNFα, IL-6, IL-10 and other chemokines), as well as faster clearance of LPS from the circulation in the PCSK9-knockout group. They further investigated the role of PCSK9 inhibition *via* a specific IgG antibody,

in addition to fluid resuscitation and antibiotics, resulting in a significant increase in survival in the treatment group after induction of polymicrobial sepsis. Moreover, they retrospectively tested their hypothesis investigating the presence of main mutations (both determining loss or gain of function) in the PCSK9 gene among 619 septic shock patients enrolled in the Vasopressin and Septic Shock Trial [96], confirming significantly lower inflammatory cytokines levels in patients with LOF allele, and underlying how patients with at least one PCSK9 LOF allele showed increased survival over a 28-day period when compared to patients without such allele or with a gain-of-function (GOF) allele. These findings related to improved survival were replicated in a second independent cohort of 415 septic shock patients, reported in the same study. In addition, the authors administered a uniform dose of LPS to healthy volunteers stratified according to PCSK9 gene mutations (110 patients with LOF *vs.* 136 patients with GOF mutations) and measured IL-6 levels at fixed timepoints, confirming a significant decrease in IL-6 levels for PCSK9 loss-of-function patients. Conversely, Berger *et al.* [27] failed to reproduce this association in an experimental study of mice with endotoxemia. The administration of alirocumab to mice after intraperitoneal LPS injection did not prove to increase survival among treated mice (independently from the timing of administration of alirocumab, even when mice were pre-treated with the drug or it was chronically administered over several weeks before experimental endotoxemia was induced) and it did not influence the levels of inflammatory markers. Moreover, no difference in mortality was noted after experimental endotoxemia in PCSK9-knockout mice *vs.* PSCK9 wild-type mice. The authors further investigated the correlation between PCSK9 levels and those of inflammatory markers among 28 human plasma samples from the Dallas BioBank collection [97] (12 of which had a PCSK9 LOF mutation) and noted no significant differences.

Further clinical evidence on the topic stems from a monocenter, observational, prospective study by Boyd and colleagues [12], in which the authors observed higher PCSK9 plasma levels in 200 patients admitted to the emergency department with suspected sepsis when compared to the values expected in healthy individuals, and that PCSK9 levels correlated with the subsequent development of cardiovascular and respiratory failure. In a prospective, monocenter study, Rannikko *et al*. evaluated plasma levels of PCSK9 in patients with microbiologically proven bacteremia [98]. The results showed increased levels of PCSK9 in this specific population, with the highest levels found in patients with lower respiratory tract infection and *Streptococcus pneumoniae* bacteremia. The authors also observed how plasma levels of PCSK9 had a direct correlation with C-reactive protein values and, above all, an indirect correlation with 7-, 28- and 90-days mortality. To support this latter finding, a retrospective analysis of 632 patients enrolled in Vasopressin and Septic Shock Trial compared to a cohort of 200 patients admitted to the emergency department with sepsis [99] reported an association between low levels of circulating LDLs and increased 28-day mortality, leading to expect a higher level of PCSK9 to be associated with higher mortality in sepsis. Surprisingly, however, in this study, higher blood levels of PCSK9 were associated with decreased mortality, supporting the conclusion that low LDL levels are unlikely to causally contribute to increased mortality in sepsis but, instead, are likely to be an indirect feature associated with the severity of sepsis. On the contrary, a retrospective evaluation of 342 patients who survived 28 days after hospital admission for sepsis and an independent validation cohort of 1079 septic shock patients admitted at the same hospital [100] showed that multiple LOF mutations in PCSK9 gene were associated with a lower risk for the composite outcome of 1-year death and infection-related readmission (HR 0.40, p=0.006), as well as lower 90-day mortality risk (OR 0.69, p=0.020). This lower mortality was confirmed in three multicenter independent cohorts (n= 170, n=130 and n=59 patients, respectively) of patients with sepsis caused by Gram-positive pathogens [101]. In this study, the authors concluded that better 28-day mortality was noted for patients with PCSK9 loss of function (73.8% *vs.* 52.8% for wild-type patients, p<0.001), and further *in vitro* data conducted on hepatocytes treated with LOF variants of recombinant PCSK9 showed an increased, dose-dependent uptake of LTA when compared to cells treated with wild-type PCSK9, possibly explaining the causal effect with the investigated clinical outcome. Recently, a large retrospective, multicenter study was conducted among a cohort of 10922 patients with an infectious disease to assess a possible association between PCSK9 genetic variants, a PCSK9 genetic risk score or genetically estimated PCSK9 expression levels and different outcomes, including the risk of development of sepsis, cardiovascular failure and in-hospital mortality. The results suggest that neither variants in the PCSK9 gene nor estimated expression of PCSK9 were associated with the risk of developing sepsis or the risk of poorer outcomes in patients admitted to hospital with an infection.

The issue of PCSK9 levels was also investigated in the subpopulation of critically ill patients admitted to the ICU. In a North-American cohort of ICU patients [25], a progressive increase in PCSK9 levels was noted over time in both septic and non-septic patients, reaching a peak at day 5 post-admission, with observed values higher than those of healthy controls. PCSK9 levels returned to normal after 14 days of ICU admission. The similar values observed among critical patients with and without sepsis suggest a lack of specificity for bacterial infection of this marker in the context of critical illness. Indeed, post-hoc analysis of blood samples collected during a randomized clinical trial [102] conducted among 150 patients with severe polytrauma assigned to randomly receive hydrocortisone or placebo, confirmed how in this specific population of ICU patients, PCSK9 levels increased progressively during admission, and they were not affected by steroid treatment. Plasma concentration of PCSK9 at day 8 post-admission was found to be positively associated with clinical severity even in the absence of infection, while levels of HDLs were negatively associated with this outcome. A recent monocenter study among 100 patients with bacterial infection admitted to the ICU for at least 48 hours [103] also aimed at investigating the impact of PCSK9 levels on antibiotic resistance or severity of disease, finding no association among the above-mentioned variables.

Given this amount of evidence, at the moment of writing, two clinical trials investigating the use of PCSK9 inhibitors evolocumab (NCT03869073) or alirocumab (NCT03634293) in the setting of sepsis are ongoing.

3.6. The Role of PCSK9 in Other Bacterial Infections

The studies carried on investigating the role of PCSK9 in bacterial infections other than sepsis or septic shock involved mainly periodontitis. Periodontitis is a highly prevalent infectious disease genetically associated with coronary heart disease [104].

In a case-control study involving 40 patients with periodontitis and 30 controls, Miyazawa and colleagues [105] observed higher PCSK9 plasma levels among periodontitis patients when compared to control subjects. This result was confirmed in another cohort of 108 Japanese patients [106], in which levels of PCSK9 and other inflammatory parameters (such as C-reactive protein, IL-6 and TNF-α, among others) were prospectively screened. A significant increase in levels of PCSK9 and C-reactive protein, as well as a reduction in total bilirubin levels, was noted among patients developing periodontal diseases compared to those who did not. Moreover, the concentration of PCSK9 was directly associated with the burden of periodontal disease.

The mechanism at the basis of this increase in serum PCSK9 levels in periodontitis remains unclear and has been investigated in some pre-clinical studies. In a mouse model of *Porphyromonas gingivalis* infection [107], an indirect role for TNF-α as an inducer of PCSK9 was proposed. Indeed, neither *P. gingivalis* infection nor the subsequent production of TNF-α directly induces PCSK9 expression, but TNF-α was found to modulate LDLR expression, with a subsequent dysregulation of lipid homeostasis that might be the driver of PCSK9 expression. In another mice study [108], the authors investigated the possible role of PCSK9 as a treatment target in a model of *P. gingivalis* infection. They observed that a loss of function in PCSK9 production reduced the release of inflammatory cytokines, such as TNF-α and IL-1β, with a concomitant enhancement in *P. gingivalis* and endotoxins clearance from the blood. However, the authors noted that periodontal bone regeneration was significantly lower in the knockout group, possibly because PCSK9 overexpression promotes osteogenesis at different levels. Further studies are needed to determine the clinical impact of PCSK9 determination in this setting and its possible implication as a treatment target. Fig. (**2**) sums up the role of PCK9 in bacterial infections.

3.7. The Role of PCSK9 in Parasitic Infections

Cholesterol is the major lipidic component of the cellular membrane of parasites, but many of them are unable to *de novo* synthesize cholesterol and they consequently exploit the host synthesis pathway to obtain the needed amount of cholesterol for membrane stability [14, 109, 110]. The hypothesis of an impact of cholesterol metabolism on the survival and virulence of parasites dates back to the 1990s. Indeed, Sein and Aikawa [111] proposed an interesting hypothesis on hypocholesterolemia as a survival advantage factor in areas where *Plasmodium falciparum* is endemic: hypocholesterolemia creates a state of mutual benefit for both the host and parasite, in which the host shows a partial immunity to infection, preventing severe manifestations, and the parasite is allowed to survive in a low proliferative state. These speculations were tested in a later *in vitro* study [112], in which infection by malaria parasites *P. falciparum* in humans and *P. yoelli* in rodents proved to be dependent on cholesterol-rich microdomains expressed on host erythrocyte or hepatocyte plasma membrane, and treatment with a specific antibody directed against these domains resulted in

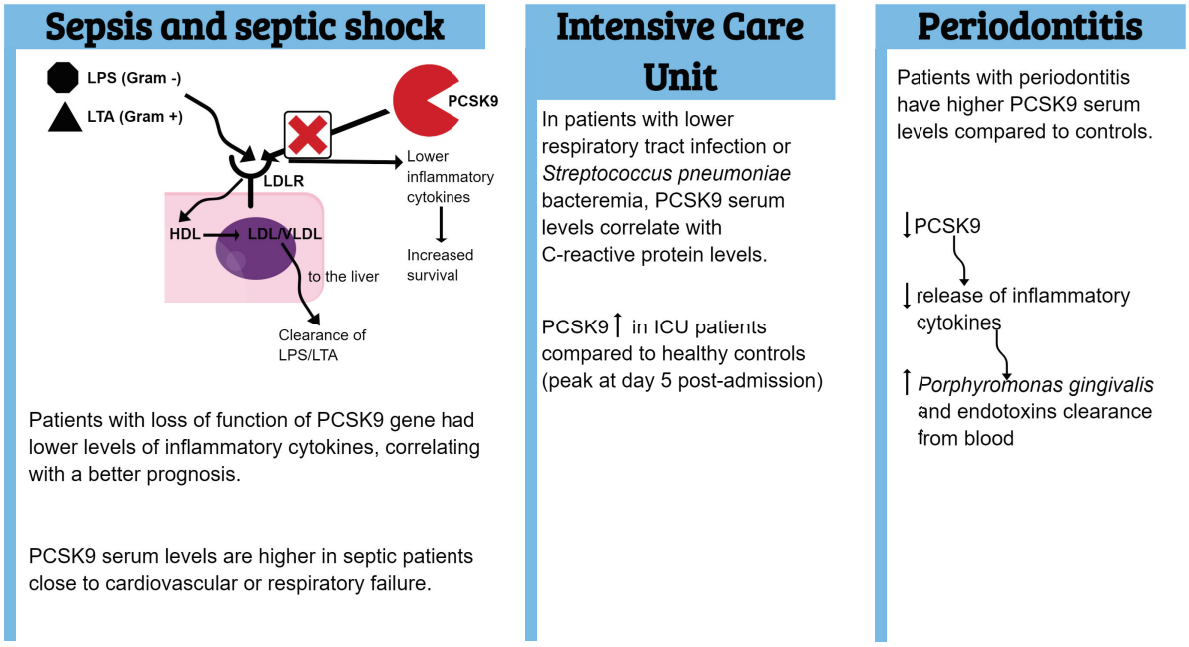

Fig. (2). Possible PCSK9 role in bacterial infections. PCSK9: proprotein convertase subtilisin/kexin type 9; LDLR: low density lipoprotein receptor; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein; LPS: lipopolysaccharide; LTA: lipoteichoic acid; ICU: Intensive Care Unit. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

reduced susceptibility to infection. Similar findings were confirmed by Samuel *et al.* [14] in a model of malarial infection, in which depletion of raft-cholesterol on erythrocyte membrane resulted in inhibition of malarial invasion by 80%. The hypothesis on the role of cholesterol in malaria infection was further carried on in another speculative paper [15], in which the authors focused specifically on PCSK9 and suggested how a loss of function of PCSK9 might be advantageous from an evolutionary point of view since it allows a more efficient uptake of cholesterol by host cells, thus "starving" parasites that depend on vascular cholesterol for proliferation and infectivity. The authors also underline how genetic mutations resulting in PCSK9 loss of function are more prevalent among populations living in tropical and subtropical regions, where parasite infections are more frequent. This latter hypothesis of a correlation between malaria severity and PCSK9 function was recently investigated in a cohort of Malian children [113], including 253 healthy controls, 246 with uncomplicated malaria and 253 with severe malaria. The authors observed a higher prevalence of homozygote GOF mutation among severe malaria patients when compared to healthy controls (3.6% *vs.* 7.9%), although they did not observe a statistically significant difference in the distribution in LOF mutations among healthy controls, as it could be expected if the mutation was protective against severe malaria. However, the authors conclude that a larger population might be needed to conclusively determine the role of LOF mutants towards malaria infection. Within the same pediatric cohort, Fedoryak *et al.* [114] reported a lower mortality (1.6% *vs.* 10.5%) among patients with severe malaria and a LOF mutation in the PCSK9 gene. Patients carrying this mutation had significantly higher levels of HDLs and lower levels of triglycerides, resulting in reduced triglycerides/HDL ratio and supporting previous observations of dyslipidemia as a risk factor for malaria severity [115].

Very few data are available on the role of lipid metabolism in parasitic infection other than malaria. Indeed, only a pre-clinical observation about the composition of *Schistosoma mansoni* membrane can be found upon review of published literature. In the paper [116], the presence of receptors able to bind human LDLs to allow the uptake of steroids on *S. mansoni* was noted and a possible role of these receptors as immune escape mediators, *via* interference with the attachment of anti-schistosomal antibodies, was postulated.

CONCLUSION

PCSK9 is a recently discovered enzyme, whose role in lipid metabolism and consequently in cardiovascular risk has been well elucidated. In this review, we focus on the interplay between PCSK9, the immune system and infectious pathogens. PCSK9 is known to upregu-

late inflammatory cytokines, but many aspects of the interaction with the immune system are still unclear. Pre-clinical studies clarified the direct role PCSK9 exerts in the pathogenesis of some infections. It is involved in reducing HCV replication, and clinical evidence confirms how higher serum levels of PCSK9 are associated with a higher probability of attainment of sustained virological response among patients treated for chronic hepatitis C. Weaker evidence is available about the role of PCSK9 in other viral infections, even if a protective role of PCSK9 inhibition has been proposed in dengue virus and SARS-CoV-2 infection.

PCSK9 is an interesting mediator of the cytokine storm typical of sepsis and septic shock. A relevant body of evidence exists about how PCSK9 plasma levels are increased in patients with sepsis, even if no clear association can be established between such a finding and clinically relevant outcomes (mortality or severity) of sepsis itself. An association between higher than usual PCSK9 levels and disease has been described in periodontitis, although again the clinical relevance of this finding has yet to be elucidated.

Of much interest is the involvement of PCSK9 in genetic susceptibility to malaria infection, suggesting that gain of function of this enzyme might predispose to severe disease, while its loss of function might result protective towards mortality.

Moreover, PCSK9 is also indirectly involved in the field of infectious diseases. This is particularly evident among people living with HIV, in whom the main interest regarding PCSK9 is linked to its atherogenic effect. Indeed, this population is particularly at risk for cardiovascular events because of a combination of factors that derive from life-style habits, antiretroviral treatment side effects and viral infection itself. Indeed, clinical trials are ongoing to investigate the effectiveness of PCSK9 inhibitors in preventing atherosclerosis among HIV-infected patients.

Despite the growing evidence on this topic, many research questions remain unanswered about the role of PCSK9 in the infectious diseases setting, although the application of PCSK9 as a target molecule seems promising in many ways. Indeed, if a role of PCSK9 in influencing morbidity and mortality will be confirmed, PCSK9 inhibitors might be a useful adjunctive therapy in the setting of sepsis and septic shock. A further clinical application of PCSK9 inhibition might lay in the prevention of malaria infection or at least in the manifestations of severe malaria, given the role that PCSK9 gain-of-function seems to play in predisposing to this parasitic disease in different studies. To conclude, PCSK9 is an interesting marker to consider in the modern management of infectious diseases and it represents a promising target for the treatment of many of such diseases.

LIST OF ABBREVIATIONS

ART	=	Antiretroviral Treatment
CXCL2	=	C-X-C Motif Chemokine Ligand 2
DENV	=	Dengue Virus
GOF	=	Gain-of-function
HCC	=	Hepatocellular Carcinoma
HCV	=	Hepatitis C Virus
HDL	=	High Density Lipoproteins
HDL-C	=	High Density Lipoproteins Cholesterol
HIV	=	Human Immunodeficiency Virus
IL-1β	=	Interleukin-1β
IL-6	=	Interleukin-6
IL-10	=	Interleukin-10
LDL	=	Low Density Lipoprotein
LDL-C	=	Low Density Lipoprotein Cholesterol
LDLR	=	Low Density Lipoprotein Receptor
LOF	=	Loss-of-function
LOX-1	=	Lectin-like ox-LDL Receptor-1
LPS	=	Lipopolysaccharide
LTA	=	Lipoteichoic Acid
miR122	=	microRNA-122
mTOR	=	Mammalian Target of Rapamycin
PCSK9	=	Proprotein Convertase Subtilisin/Kexin type 9
PLWHIV	=	People Living with HIV
SARS-CoV-2	=	Severe Acute Respiratory Syndrome Coronavirus-2
SKI-1/S1P	=	Subtilisin/Hexin-isoenzyme-1/site-1
SVR	=	Sustained Virological Response
TLR	=	Toll-like Receptor
TLR4/NF-κB	=	Toll-like Receptor 4/Nuclear Factor-κB
TNF-α	=	Tumor Necrosis Factor- α
VLDL	=	Very-low-density Lipoproteins

VSMCs = Vascular Smooth Muscle Cells

AUTHORS' CONTRIBUTIONS

Laura Magnasco, Chiara Sepulcri, Roberta Maria Antonello, and Laura Labate drafted the first version of the paper. Matteo Bassetti and Daniele Roberto Giacobbe, significantly contributed to the design and idea. All the authors (Laura Magnasco, Chiara Sepulcri, Roberta Maria Antonello, Stefano Di Bella, Laura Labate, Roberto Luzzati, Daniele Roberto Giacobbe and Matteo Bassetti) significantly contributed to the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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