View of statins as antimicrobials in cardiovascular risk modification

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Abstract
Atherosclerosis is a complex arterial pathological development underlying heart attack and stroke and a leading cause of death in developed and now also in developing countries. The primary processes that lead to the inflammatory lipid-laden proliferative lesion, obstructing the blood flow, and referred to as atherosclerotic plaque are dyslipidaemia and inflammation. Here, we will review one of the most efficient classes of drugs indicated for management of cardiovascular disease (CVD), statins. We will assess their pleiotropic effects that emerged from CVD applications, focusing this review specifically on plausible antimicrobial activity. Only recently gaining strength, the recognition of possible antibacterial activity may extend the statin applicability for vascular as well as to other critical inflammatory conditions.

Keywords
Atherosclerosis • Statins • Microbial infection • Inflammation

1. Atherosclerosis: statins in cardiovascular disease
Atherosclerosis (AS), a chronic inflammatory disease, is the most common cause of cardiovascular disease (CVD), the primary cause of morbidity and mortality in adults. Statins, competitive 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are among the most prescribed medications for the prevention of CVD. Statins lead to reduced liver cholesterol synthesis by mediating the inhibition of HMG-CoA reductase, the enzyme which catalyses the reduction of 3-hydroxy-3-methylglutaryl-CoA to L-mevalonic acid in cholesterol biosynthesis.1,2 Since statin molecule is structurally similar to HMG-CoA, statins compete for the enzyme-binding site as competitive antagonists of HMG-CoA reductase. This inhibition reduces the mevalonate synthesis, a rate-limiting step in the cholesterol biosynthesis pathway involving 25 enzymes, and subsequently, the cholesterol synthesis rate.

Primary and secondary prevention along with acute statin therapy of CVD patients has demonstrated clinical benefits.3,4 A multicentre study on prospectively collected data of 2072 stroke patients concluded that statins administered in the acute phase of stroke may improve short- and long-term outcomes.5 The use of high-dose statins in conjunction with percutaneous coronary intervention with stent implantation (PCI and coronary angioplasty) has also shown a significant synergistic effect and safety by reducing ischaemia and necrosis in patients with severe coronary disease.6

Intravascular ultrasound imaging end point studies with statins have shown that they reduce atherosclerotic plaque burden in treated patients,7 and the analysis by magnetic resonance imaging of aortic and carotid artery plaques of patients treated with simvastatin has shown that statins reduced the size of the lesions and the thickness of the arterial wall without changes in the lumen size.8 Also, recent clinical studies reported changes in carotid artery morphology in terms of increasing echogenicity and fibrous tissue content as an effect of statins.9–12

In the USA alone, approximately 32 million individuals take statins to lower plasma cholesterol levels.13 The emergence of several pharmaceuticals in this class is due to their evidence-based efficacy in lowering cardiovascular risk.14

2. Infectious component of AS
2.1 Risk factors
The ‘classical’ atherogenic risk factors such as smoking, hypercholesterolaemia, hypertension, and hyperglycaemia can trigger endothelial activation manifested by the expression of endothelial cell adhesion molecules, which leads to adhesion of monocytes to the arterial wall.15 Although it has been shown that the these risk factors are almost always present among individuals with clinical CVD, the incidence of AS, the so-called ‘residual risk’, is not fully explained by conventional risk factors.16 Myocardial infarction (MI) and stroke continue to occur in up to two-thirds of all patients on statins, the ‘forgotten
It has also been shown that 60–70% of individuals with multiple CVD risk factors have not experienced a cardiovascular event.

While the classical risk factors might be necessary for initiation and progression of AS, they may not be sufficient. Similar to other chronic infections, AS has all features of an inflammatory condition. A recent large genome-wide association study identified both inflammation and lipid metabolism as key biological pathways involved in the genetic pathogenesis of coronary artery disease (CAD), bringing strong new molecular evidence in support of the causal involvement of inflammatory mechanisms in the pathogenesis of coronary AS. This turned the attention to infections as a potential causative factor.

### 2.2 Infectious component: evidence for the hypothesis that bacterial infections cause CVD

Indeed, the notion that pathogens are responsible for chronic conditions dates back to the 1880s when *Streptococcus* and *Salmonella* were shown by French investigators to induce atherosclerotic plaques in rabbits. There is accumulating epidemiological evidence in support of this idea. AS does have many of the characteristics of a chronic inflammatory disease with an abundance of epidemiological and seroepidemiological data to support the view that infections contribute to AS.

Multiple animal studies also pointed in that direction. For example, oral infection with *Porphyromonas gingivalis*, main periodontal pathogen, accelerated early AS in apolipoprotein E (ApoE)-null mice. Metronidazole-treated ApoE(+/−) mice developed significantly fewer atheromatous lesions in the proximal aorta and the aortic tree compared with animals injected with wild-type *P. gingivalis*. Furthermore, rabbits with experimentally induced periodontitis had more extensive lipid accumulations in the aorta than did non-diseased animals (P < 0.05). Producing similar results in large animals, intravenous injections with *P. gingivalis*, designed to mimic periodontitis-associated bacteremia, promoted coronary artery and aortic lesions in normocholesterolaemic pigs, and increases aortic and coronary AS in hypercholesterolaemic pigs. *Streptococcus mutans*, a main causative agent of dental caries and endocarditis, as well as polymicrobial infection with major periodontal pathogens was also associated with atherogenesis in ApoE-null mice. In terms of mechanism of pathogen-induced atherogenesis, it is also important to point that ApoE(+/−) mice injected with invasion-deficient *P. gingivalis* mutant showed significantly fewer atheromatous aortic lesions, compared with animals injected with wild-type bacteria.

In humans, the Northern Manhattan Study (NOMAS), a prospective cohort study of stroke incidence and prognosis, showed that the infectious burden in stroke-free individuals is associated with the risk of stroke and carotid plaque thickness. The infectious burden index was associated with an increased risk of all strokes [adjusted hazard ratio (HR) 1.39; 95% confidence interval (CI) 1.02–1.90] after adjusting for demographics (including education) and risk factors (including smoking). Similar results were obtained when the individuals with coronary disease were excluded (adjusted HR 1.50; 95% CI 1.05–2.13). Furthermore, the Oral Infections and Vascular Disease Epidemiology Study (INVEST) found significant association between tooth loss and carotid plaque prevalence. Among those with 0–9 missing teeth, 46% had carotid artery plaque, whereas among those with ≥10 missing teeth, carotid artery plaque prevalence was ∼60% (P < 0.05). Adjustment has been made for conventional risk factors including smoking, race-ethnicity, education, physical activity, healthy lifestyle, and psychosocial health. INVEST also demonstrated that colonization of periodontium by pathogenic periodontal pathogens is associated with increased carotid artery intima-media thickness (IMT). Multiple adjustments have been made, including for race/ethnicity, education, and smoking. In this study of 657 dentate subjects, 11 known periodontal bacteria were quantitatively assessed by DNA hybridization in 4561 sub-gingival plaque samples. The results showed that causative periodontal bacterial burden was related to carotid IMT.

Extended reviews on periodontitis and CVD are available elsewhere. Detailed information on bacterial *in vitro* and *in vivo* studies related to CVD have been recently published.

### 2.3 Indirect mechanisms by which infection may contribute to AS

Biological mechanisms specifically linking periodontal infection and AS are summarized in Figure 1. *Porphyromonas gingivalis* infection has been related to an increase incidence of macrophages, T cells, and lipids within the plaques, and certain strains of *P. gingivalis* have demonstrated the ability to infect macrophages and enhance foam cell formation in the vascular wall along with accelerating lesion development, in part, via a Toll-like receptor (TLR)2-mediated mechanism. There is also evidence that periodontal pathogens, more specifically *P. gingivalis*, induce expression of adhesion molecules [intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin] and stimulate monocyte adhesion to cultured human endothelial cells. In addition, increased activation of platelets has been reported in subjects with periodontitis and infection with periodontal pathogen, *P. gingivalis* in a rat model has shown to induce platelet activation and increase aggregation in whole blood.

Furthermore, an increase in inflammatory biomarkers, such as high-sensitivity C-reactive protein (hsCRP), was found to predict acute events in healthy individuals. In fact, systemic manifestations of periodontal infections can present as increased levels of hsCRP, fibrinogen, tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and other acute phase reactant proteins associated with cardiovascular events. The AFCAPS/TexCAPS trial, persons with low low-density lipoprotein (LDL) cholesterol and elevated hsCRP had a substantial benefit from lovastatin. This finding was one of the justifications for the large JUPITER trial, involving more than 1000 physicians in 26 countries and 17 802 apparently healthy individuals. In this trial, the rates of the combined primary endpoint (MI, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes) were 0.77 and 1.36 per 100 person-years of follow-up in the rosuvastatin and placebo groups, respectively. JUPITER demonstrated clinical benefit in CVD-free individuals due to both decreasing of LDL level and anti-inflammatory activity as reflected by decreased CRP level. Accordingly, hsCRP concentration has continuous associations with the risk of vascular mortality [risk ratio 1.55 (95% CI 1.37–1.76)]. Therefore, identification of other inflammatory biomarkers such as hsCRP should help to improve the classical risk factor-based CVD risk assessment.

Another possible mechanism for an indirect effect connecting periodontal infection and CVD is the molecular mimicry or cross-reactivity between antibodies specific for bacterial proteins and host antigens such as heat shock proteins (HSP60), which was shown to lead to vascular inflammation and AS.
2.4 Direct infection of vascular cells

In concordance with the proposed infectious component of vascular inflammations, live invasive periodontal bacteria were identified in atheromatous tissue from a patient.53 DNA analysis data also suggest that a variety of pathogens are associated with atherosclerotic tissue. 54– 56 *Chlamydophila pneumoniae* was isolated from atheromatous tissues in the 1990s,57 and more recently, we identified and cultivated from atheromas a variety of bacterial pathogens,58 bringing forward the subject of statins as antimicrobials and providing a legitimate target for novel antimicrobial and anti-inflammatory therapeutics. In fact, the latest genome-wide association network analysis of 63,746 CAD cases and 130,681 controls identified lipid metabolism and inflammation as the two key biological pathways involved in the genetic predisposition to coronary disease.19 A summary of the current evidence supporting an infection-based model of atherogenesis has been reported59– 61 (Figure 2), bridging fundamental and clinical research data.

2.5 The quest for causality: clinical interventions

The abundant communications linking infectious organisms with CV inflammations, however, are insufficient to make a clear transition from mere association—to so-called ‘innocent bystander’ present at the site—to causality, where the organism fulfils the Koch’s postulate. In addition, the initiating agent early in the process of atherogenesis could hardly be identified since this stage is asymptomatic.62 Further, atherogenesis occurs in response to a variety of stimuli. Nevertheless, prompted by the evidence of *C. pneumoniae* in atheromatous tissue and by promising pilot studies (that however did not account for confounders or did not have enough power),63 several large-scale randomized prospective controlled clinical trials were conducted last decade to test the hypothesis that the infections are another underlying risk factor on atherogenesis. The results were disappointing in that in all large trials, short- and long-term antibiotic treatments were not found to prevent the outcome events as hypothesized.

Specifically, the ACADEMIC azithromycin treatment trial of 302 CAD patients seropositive to *C. pneumoniae* showed no significant difference in the primary end point between the antibiotic and control groups (HR for azithromycin 0.89; 95% CI 0.51–1.61; *P* = 0.74).64 Furthermore, a roxithromycin tertiary prevention trial of 872 MI patients showed 6.5% total mortality (28 of 431) in the roxithromycin group compared with 6.0% (26 of 437) in the placebo group at 12 months [odds ratio (OR) 1.1; 95% CI 0.6–1.9; *P* = 0.739].65 Importantly, in the WIZARD azithromycin trial of 7747 MI patients that had an elevated *C. pneumoniae* IgG titre, there was no significant risk reduction in the likelihood of a primary event with antibiotic vs. placebo (7% [95% CI 2 to 17%], *P* = 0.23).66 Again, no significant risk reduction of coronary events was detected in a randomized, prospective trial of 4012 patients for secondary prevention in the azithromycin group when compared with the placebo group with regard to the primary endpoint (risk reduction, 1% [95% CI –13 to 13%]).67

Similarly, addressing peripheral vascular disease (PAV) offered no benefits for survival or ankle pressure. In the randomized clinical trial of 509 patients for secondary prevention of AS through *C. pneumoniae* eradication (SPACE trial), the treatment was a short-term course of azithromycin. The number of complications (131 in the treatment group vs. 121 in the placebo group) and the number of patients who developed complications [98 (38%) in the azithromycin vs. 84 (33%) in the placebo group] were comparable in both groups.68 Again in PAV, a roxithromycin trial of 507 patients yielded no significant differences between treatment and placebo groups. The unadjusted HR of death from all causes was 1.13 (95% CI 0.68–1.90) and of primary events 0.92 (95% CI 0.67–1.26); no significant differences were found on secondary events. Primary events were death, peripheral revascularization, and major lower limb amputation and secondary events were thrombosis, stroke, transient cerebral ischaemic attack, and MI.69 An extended list of statin clinical trials is presented in Table 1.
Nevertheless, the following weaknesses of the clinical trial designs must be taken into account, leaving open the door to further testing of the hypothesis.

To begin with, the \textit{C. pneumoniae}-targeting trials were not designed to affect intracellular \textit{C. pneumoniae} forms, which are drug-resistant and cannot be eliminated by azithromycin.\cite{90} In addition, the target agent, \textit{C. pneumoniae}, was only rarely isolated in culture from atherosclerotic tissue.\cite{91} Even worse, many antibiotics at sub-inhibitory concentrations induce \textit{C. pneumoniae} persistence.\cite{92} Next, inconclusive results only mean that certain drug treatment has no effect on the trial outcome measures. Another weakness of the null results at advanced stage of disease is that the treatment might have been successful at earlier stages. Furthermore, the evidence suggests the infection that takes place in the atheromata is polymicrobial,\cite{22,59} thus the antibiotic treatment trials would not be expected to be efficient against all pathogenic species; therefore, the treatment might have missed most targets altogether. Notably, there was a significant association between infectious burden and the extent of AS, where the risk for future death was increased by the number of infectious pathogens, especially in patients with advanced AS.\cite{93} In fact, even before the outcome of these trials was known, there has already been recognition that ‘even these randomized trials are likely to be only partially informative’.\cite{94}

Ultimately, only a long prospective antibiotic treatment trial of a very large patient cohort controlled for the other risk factors could definitely answer the causality question. However, the prospect of having a large segment of the population on continuous antibiotic therapy for a prolonged period of time is clearly not acceptable for variety of reasons, mainly the prospect of increased microbial drug resistance.

In summary, endothelial injury and inflammation, monocyte influx and sub-endothelial retention and oxidation and accumulation of LDL particles in the tunica intima are the central pathogenic event that promotes atherosclerotic lesion formation.\cite{95,96} Since infection can lead to an accelerated atherogenesis, cholesterol-induced intimal thickening, and foam cell accumulation,\cite{97} here we will explore the current knowledge regarding the effect of statins on the infectious component of atherogenesis.

3. Are statins anti-bacterials?

3.1 Evidence that statin treatment affects bacterial infections

Since the introduction of statins in 1987 (Merck’s lovastatin), multidimensional investigations into the effect of statin therapy on AS patients have been carried out. This has led to clarification of the mechanism of action and, due to the low toxicity of statins, to the search for additional physiological effects.

Interestingly, clinico-epidemiological studies on the effects of statins on CVD-related morbidity and mortality demonstrated cholesterol-independent pleiotropic effects such as improvement of endothelial
Aspergillus can be synergistic with various azole anti-fungals.\textsuperscript{107} *Enterococcus* species and fungi. Statins inhibited virulence-mitigating and inflammation-suppressing moieties against bacterial infections.\textsuperscript{98–102} In vitro studies have hinted at HMG-CoA reductase inhibitors as immunity modulation.\textsuperscript{98–102} Furthermore, the activity was present against clinical isolates of *C. pneumoniae*.\textsuperscript{104} However, likely due to the differences in enzyme targets and between gram-negative and -positive bacteria, fungi, or parasites, such results are inconsistent and need detailed confirmation.\textsuperscript{109} The recently reported lack of inhibition of several pathogens (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *Escherichia coli*) by lovastatin, fluvastatin, atorvastatin, pravastatin, and simvastatin also suggest an effect on the host.\textsuperscript{110}

### Table 1 Clinical trials of antibiotic treatment of patients for secondary prevention of atherothrombotic vascular disease

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Statin trial, Ref #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gupta</td>
<td>1997</td>
<td>An increased anti-Cp antibody titre may be a predictor for further adverse events\textsuperscript{70}</td>
</tr>
<tr>
<td>Gurfhinkel</td>
<td>1999</td>
<td>Roxithromycin in non-Q-wave coronary syndromes: ROXIS pilot Study\textsuperscript{71}</td>
</tr>
<tr>
<td>Anderson</td>
<td>1999</td>
<td>The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) study\textsuperscript{72}</td>
</tr>
<tr>
<td>Muhleistten</td>
<td>2000</td>
<td>Azithromycin therapy is not associated with marked reductions in ischaemic events\textsuperscript{64}</td>
</tr>
<tr>
<td>Mosorin</td>
<td>2001</td>
<td>Doxycycline may favourably alter the outcome of patients with small AAA\textsuperscript{73}</td>
</tr>
<tr>
<td>Neumann</td>
<td>2001</td>
<td>Roxithromycin after coronary stenting: (ISAR-3) trial\textsuperscript{74}</td>
</tr>
<tr>
<td>Leowattana</td>
<td>2001</td>
<td>Roxithromycin does not reduce acute coronary syndrome associated with <em>C. pneumoniae</em> infection\textsuperscript{75}</td>
</tr>
<tr>
<td>Parchure</td>
<td>2002</td>
<td>Azithromycin in patients with coronary artery disease and evidence of <em>C. pneumoniae</em> infection\textsuperscript{63}</td>
</tr>
<tr>
<td>Wiesli</td>
<td>2002</td>
<td>Roxithromycin prevents PAD progression in <em>C. pneumoniae</em> seropositive men\textsuperscript{76}</td>
</tr>
<tr>
<td>Sander</td>
<td>2002</td>
<td>Roxithromycin reduces progression of early carotid AS in <em>C. pneumoniae</em> seropositive patients\textsuperscript{77}</td>
</tr>
<tr>
<td>Sinisalo</td>
<td>2002</td>
<td>Effect of 3 months of clarithromycin in acute non-Q-wave coronary syndrome\textsuperscript{78}</td>
</tr>
<tr>
<td>Stone</td>
<td>2002</td>
<td>South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA)\textsuperscript{79}</td>
</tr>
<tr>
<td>Da Costa</td>
<td>2003</td>
<td>Roxithromycin showed no effect on early thrombotic events after coronary stent placement\textsuperscript{80}</td>
</tr>
<tr>
<td>O’Connor</td>
<td>2003</td>
<td>A 3-month course of azithromycin did not significantly reduce the clinical sequelae of CAD in MI patients with <em>C. pneumoniae</em> exposure\textsuperscript{84}</td>
</tr>
<tr>
<td>Cercek</td>
<td>2003</td>
<td>Azithromycin in Acute Coronary Syndrome (AZACS) trial\textsuperscript{81}</td>
</tr>
<tr>
<td>Zahn</td>
<td>2003</td>
<td>Roxithromycin after acute MI did not reduce event rates during 12 months of follow-up\textsuperscript{65}</td>
</tr>
<tr>
<td>Burkhardt</td>
<td>2004</td>
<td>Roxithromycin therapy in MI patients: ANTIBIO trial\textsuperscript{82}</td>
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<tr>
<td>Hillis</td>
<td>2004</td>
<td>Azithromycin in survivors of an acute coronary syndrome\textsuperscript{83}</td>
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<tr>
<td>Krayerbuehl</td>
<td>2005</td>
<td>Clarithromycin inhibits PAD progression associated with <em>C. pneumoniae</em>\textsuperscript{84}</td>
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<tr>
<td>Berg</td>
<td>2005</td>
<td>Clarithromycin prior to coronary artery bypass graft does not prevent subsequent cardiac events\textsuperscript{85}</td>
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<tr>
<td>Vainas</td>
<td>2005</td>
<td>SPACE: a secondary prevention azithromycin randomized clinical trial in PAD patients\textsuperscript{68}</td>
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<tr>
<td>Grayston</td>
<td>2005</td>
<td>A 1-year azithromycin did not alter cardiac events among patients with stable CAD\textsuperscript{87}</td>
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<td>Joensen</td>
<td>2008</td>
<td>Long-term roxithromycin is ineffective in preventing death and acute events in PAD patients\textsuperscript{88}</td>
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<tr>
<td>Karlsson</td>
<td>2009</td>
<td>Azithromycin did not have any effect on AAA expansion\textsuperscript{86}</td>
</tr>
<tr>
<td>Takagi</td>
<td>2010</td>
<td>Statin therapy is associated with less expansion rates in patients with small AAA\textsuperscript{87}</td>
</tr>
<tr>
<td>Takagi</td>
<td>2012</td>
<td>Statin therapy prevents growth of small AAAs, more beneficial for larger AAA diameter\textsuperscript{88}</td>
</tr>
<tr>
<td>Takagi</td>
<td>2013</td>
<td>0.63 mm/year reduction in AAA growth rates with statin therapy\textsuperscript{89}</td>
</tr>
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Cp, *C. pneumoniae*; MI, myocardial infarction; CAD, coronary artery disease; PAD, peripheral artery disease; AAA, abdominal aortic aneurysm.

The clinical data in the case of methicillin-susceptible and -resistant *S. aureus* (MSSA + MRSA) were also tested in vitro using the microtitre dilution method. Simvastatin showed a significant antimicrobial effect against MSSA (mean MIC 29.2 mg/L) and to a lesser extent against MRSA (mean MIC 74.9 mg/L).\textsuperscript{104} However, likely due to the differences in enzyme targets and between gram-negative and -positive bacteria, fungi, or parasites, such results are inconsistent and need detailed confirmation.\textsuperscript{109} The recently reported lack of inhibition of several pathogens (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *Escherichia coli*) by lovastatin, fluvastatin, atorvastatin, pravastatin, and simvastatin also suggest an effect on the host.\textsuperscript{110}

### 3.2 In vitro investigations

*In vitro* studies have hinted at HMG-CoA reductase inhibitors as virulence-mitigating and inflammation-suppressing moieties against bacteria and fungi. Statins inhibited *in vitro* virulence phenotypes of *Pseudomonas aeruginosa*\textsuperscript{103} and showed direct pathogen activity against several strains of *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci.\textsuperscript{104} Furthermore, the activity was present against clinical isolates of *Aspergillus*\textsuperscript{105} and *Plasmodium falciparum*.\textsuperscript{106} The antifungal activity can be synergistic with various azole anti-fungals.\textsuperscript{107}

In a study of the *in vitro* and *in vivo* effect of simvastatin on *Streptococcus pneumoniae* and *Moraxella catarrhalis*, it was observed that, at high concentrations (36 µmol/L), simvastatin rapidly kills the bacteria. However, these concentrations by far exceed the concentrations detected in human blood during simvastatin therapy (1–15 nmol/L) and single doses of statins given to healthy volunteers [simvastatin, 80 mg (n = 3); fluvastatin, 40 mg (n = 1); and penicillin (Kavepenin), 1 g (n = 1)] did not improve antibacterial effects of whole blood.\textsuperscript{108}

### 3.3 In vivo investigations

In a murine model of acute *C. pneumoniae* infection, simvastatin treatment showed both anti-chlamydial and immunomodulatory effects during infection.\textsuperscript{111} Statins suppress bacterial *Anaplasma phagocytophilum* infections in an ApoE-null murine model via lipid modifications, since plasma lipids facilitate this infection, and also up-regulate a pro-inflammatory chemokine (macrophage inflammatory protein, MIP-2) and its receptor, CXCR2.\textsuperscript{112} Pre-treated with statins before infection with *Mycobacterium tuberculosis* mice displayed increased host function and plaque stability, decreased oxidative stress and inflammation, reduced inflammation and thrombogenic response, and innate immunity modulation.\textsuperscript{98–102}
protection, reduced lung burdens, and improved histopathological findings.\textsuperscript{113} Furthermore, simvastatin modulated the inflammatory mediators elicited by Trypanosoma cruzi and ameliorated the heart damage in a murine model of Chagas disease.\textsuperscript{114} Similarly, the murine model has been used to observe antifungal activity\textsuperscript{115,116} and anti-malaria activity (in combination with mefloquine),\textsuperscript{117} but has not been conclusive for influenza A treatment.\textsuperscript{118}

### 3.4 Application of statins in bacteraemia

Bacteraemia is the condition when culturable bacteria are found in the bloodstream. It may occur through a wound infection or during surgery, may be symptomatic or asymptomatic, and may lead to cardiac and vascular inflammations.

In a retrospective review of 388 Gram-negative bacilli and S. aureus-related bacteraemic infections, a significant reduction in both overall (6 vs. 28%; $P = .002$) and attributable (3 vs. 20%; $P = .010$) mortality was seen among patients taking statins compared with patients not taking statins. This reduction, attributable to anti-microbial activity, was confirmed in a multivariate analysis (OR 7.6: 95% CI 1.01–57.5).\textsuperscript{119} Recently, in a study of 133 CVD patients, 47 blood samples (35.3%) were found culture positive, indicating a high level of bacteraemia in these patients. Fifty-seven bacterial isolates from 35 different species were further identified in the positive specimens, with coagulase-negative Staphylococci the most predominant species.\textsuperscript{120} Bacteraemias can also lead to septic shock.

A study examining LPS-induced shock, pre-treatment of healthy volunteers with simvastatin reduced LPS-induced bronchoalveolar lavage fluid neutrophilia, myeloperoxidase, TNF-$\alpha$, MMP-7, 8, and 9, and CRP ($P < 0.05$ vs. placebo).\textsuperscript{121}

To explore the immunomodulatory effect of statins, 27 healthy volunteers were given simvastatin or atorvastatin for 14 days. Serum cytokines and acute phase proteins along with HLA-DR and CD38 expression on T-cells and superantigen-mediated T-cell activation ex vivo before and after treatment were analysed. A different immunomodulatory effect of the two statins on human T-cells was observed, with simvastatin-mediated inhibition of the superantigen-mediated T-cell activation. This might underlie the reduced mortality of simvastatin-treated patients with staphylococcal bacteraemia.\textsuperscript{122} In that vein, an investigation of the anti-inflammatory effect of statin pre-treatment on healthy volunteers, who were then administered intravenous LPS, high-dose simvastatin pre-treatment blunted monocye TLR4 and TLR2 expression. The suppressive effect of statins on key innate immunity receptors in this human endotoxaemia model was associated with a reduction of effector cytokines.\textsuperscript{123}

In a retrospective study of 319 bacteraemic individuals divided according to statin use and duration of therapy prior to the bacteraemic episode, the absence of statin use in bacteraemic patients was associated with increased 30-day all-cause mortality (HR = 2.98; 95% CI 1.59–5.56, $P = 0.001$).\textsuperscript{124} Pneumococcal pneumonia patients who received statins at time of admission had better clinical outcomes than those who did not; concurrent treatment with a macrolide did not appear to confer an additional survival benefit.\textsuperscript{125} Statin use before diagnosis of community-acquired pneumonia (CAP), the most common cause of death from infection, is associated with an improved outcome.\textsuperscript{126} Such treatment may modestly reduce the incidence of pneumonia as well, as seen from the analysis of data from 17 802 healthy participants in the JUPITER trial.\textsuperscript{127} Statins’ pleiotropic properties may also provide protection against Clostridium difficile infection.\textsuperscript{128}

### 3.5 Application of statins in sepsis and other infections

Sepsis is a life-threatening condition despite the medical advances used for its treatment. It is a systemic immune system response to a focal infection that can cause widespread tissue damage and organ failure, with 29% fatality in severe sepsis.\textsuperscript{129} In the USA, sepsis is among the leading causes of death for intensive care patients and accounts for 9% of the overall annual mortality with 44 deaths per 100 000 people.\textsuperscript{130–132}

To test whether statin administration before a sepsis-inducing insult reduces morbidity and improves survival, half of 69 168 patients older than 65 years hospitalized for an MI, stroke, or revascularization were administered a statin and half were not. The incidence of sepsis was lower in patients receiving statins than in controls (HR 0.81; 95% CI 0.72–0.91).\textsuperscript{3,133–135} In severe sepsis rat models, after cecal ligation and puncture surgery, treatment with statins showed a benefit in improving survival.\textsuperscript{136} According to other studies, however, no statistically significant association between statin therapy before bloodstream infection and survival was identified.\textsuperscript{137}

A study was carried out to assess the effects of adding simvastatin to a triple drug regimen in patients with Helicobacter pylori infection and if this addition will improve the eradication rate.\textsuperscript{138} The eradication rates were higher with simvastatin than with placebo ($P = 0.03–0.04$), demonstrating that simvastatin as adjuvant to standard therapy may significantly improve the H. pylori eradication rate.

### 3.6 Statins in periodontitis

Important evidence solidifying the link between fundamental and applied research has been obtained for periodontitis, a chronic inflammatory infection. An investigation assessing the periodontal status of hyperlipidaemic patients on statins concluded that (i) patients with hyperlipidaemia are more prone to periodontitis, and that (ii) statins can be beneficial for periodontal health. This cross-sectional study of 94 patients with hyperlipidaemia (50 receiving statins and 44 receiving non-pharmacological therapy) and 46 controls found that two key clinical measures of periodontal disease (gingival index [GI] and probing depth [PD]) were significantly higher in patients with hyperlipidaemia who were non-statins users compared with the normallipolaemia individuals ($P < 0.001$ [PD] and $P < 0.05$ [GI]) and the statin-treated patients ($P = 0.001$ [PD] and $P < 0.05$ [GI]). Total cholesterol (TC) and serum triglyceride were found to correlate significantly with periodontitis ($P < 0.001$) and with GI, respectively ($P = 0.020$).\textsuperscript{139}

Additional findings suggesting the effect of statins in chronic periodontal inflammations was demonstrated in a trial of 83 adults with risk factors or with established AS, randomized to atorvastatin 80 vs. 10 mg. The evaluation of the impact of atorvastatin on arterial inflammation was performed using fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT). The impact of high-dose atorvastatin was greatest in individuals with evidence of active periodontitis, and was evident after a 4-week treatment period. After 12 weeks, there was a significant reduction in periodontal inflammation in patients randomized to atorvastatin 80 vs. 10 mg ($\Delta$TBR mean [95 CI%], 80 vs. 10 mg group = $-0.43$ [−0.83 to −0.02], $P = 0.04$).\textsuperscript{140} Most notably, the changes in periodontal inflammation correlated with changes in carotid inflammation ($R = 0.61$, $P < 0.001$). Thus, the overall beneficial effect of statins against various infections includes a positive effect on periodontal health. The main references in the area of antibacterial applications of statins are listed in Table 2.
4. Possible mechanisms of antimicrobial activity

4.1 Interference with L-mevalonic acid synthesis

Direct antimicrobial activity is not proved; therefore, the activity seems to stem from statins’ effect on host cells. Statins have been shown to lower LDL levels by interfering with mevalonate synthesis; however, the reduction of mevalonate levels has a potential to influence pathogen-induced inflammations as well. Mevalonate inhibition can affect cell signalling pathways and thus diminish plasma markers of inflammation, T-cell and monocyte activation, and blood clotting. This inhibition has also been shown to reduce the inflammatory factor-driven cholesterol and free radical production in endothelial cells. In a new finding, metabolic rescue experiments demonstrated that statins reduce membrane cholesterol, specifically by the mevalonate–isoprenoid arm of the sterol pathway, promoting phagosomal autophagy in macrophages (LC3-II). These results suggest that the cholesterol reduction mediated by statins is of importance for the protection of host cells from bacterial infections. The mechanism of simvastatin-mediated protection from LPS-induced inflammation, resulting in a reduction of acute lung injury unaffected. The mevalonate inhibition mediates reduction in the synthesis of isoprenoid farnesyl pyrophosphate and geranylgeranyl pyrophosphate that, in turn, leads to modulation of post-translational prenylation of Ras, Rho, and Rac. Since the latter regulate the homeostasis at variety of levels, such as endothelial function, plaque stability, platelet activity, coagulation, oxidation, and inflammatory responses, this is another avenue for mevalonate inhibition to display beneficial effects with statin-treated CVD patients.

4.2 COX-2 modulation

Some of the myocardial protective effects of statins can also be explained with modulation of prostaglandin G/H synthase-2 (cyclooxygenase-2, COX-2). It has been shown that inhibition of COX-2 leads to elevated risk of MI and stroke. The atorvastatin-mediated enhancement of COX-2 stability could increase dendritic cell function after infectious bouts and could also counteract some of the untoward effects associated with sustained inhibition of COX-2.

4.3 Reactive oxygen species suppression

Simvastatin has recently shown benefits in a model of LPS-induced lung inflammation, resulting in a reduction of acute lung injury unaffected. In that study, treatment before and after onset of acute lung injury reduced neutrophil influx into the lung as well as lung permeability, thus indicating a protective role of simvastatin in lung inflammations. The mechanism of simvastatin-mediated protection from LPS-induced acute lung injury seems to involve reduced formation of reactive oxygen species (ROS), oxidation of LDL, and adhesion of neutrophils, while apoptosis, bacterial phagocytosis, and bacterial clearance remain unaffected. In terms of ROS formation, it is Rac-1 binding to p67phox that activates the NADPH oxidase system and the ROS generation. As mentioned above, statins block Rac, suppressing Rac-1-mediated NADPH oxidase-dependent generation of ROS.

It seems that suppression of ROS formation is required for reduced neutrophil adhesion and recruitment. For example, production of superoxide and its derivative, hydrogen peroxide by neutrophils was increased in women with preeclampsia. In the same time, neutrophils from women with preeclampsia demonstrate increased CD11b expression and adhesion to endothelial cells, likely caused by the excess superoxide. In addition, suppressing ROS can protect intercellular tight junctions of the

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Table 2 Recent contributions in the area of antibacterial applications of HMG-CoA reductase inhibitors

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Statin activity</th>
<th>Ref #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fehr</td>
<td>2004</td>
<td>Reduced mortality in simvastatin-treated patients with staphylococcal bacteraemia.</td>
<td>122</td>
</tr>
<tr>
<td>Niessner</td>
<td>2006</td>
<td>Anti-inflammatory effect of simvastatin in the human endotoxaemia model</td>
<td>123</td>
</tr>
<tr>
<td>Hackam</td>
<td>2006</td>
<td>Significant reductions in severe and fatal sepsis in high-risk patients with diabetes mellitus, chronic renal failure, or a history of infections</td>
<td>133</td>
</tr>
<tr>
<td>Shyamsundar</td>
<td>2009</td>
<td>Simvastatin reduces LPS-induced bronchoalveolar inflammation</td>
<td>121</td>
</tr>
<tr>
<td>Calisto</td>
<td>2010</td>
<td>Atorvastatin improves survival in the animal model of severe sepsis: effect on tissue inflammatory pathway and on insulin signalling</td>
<td>136</td>
</tr>
<tr>
<td>Bergman</td>
<td>2011</td>
<td>Antibacterial activity is not due to direct bactericidal effect: in vitro and in vivo studies</td>
<td>108</td>
</tr>
<tr>
<td>Nseir</td>
<td>2012</td>
<td>Simvastatin as adjuvant improves H. pylori eradication rate</td>
<td>138</td>
</tr>
<tr>
<td>Wunderink</td>
<td>2012</td>
<td>Statin use before CAP diagnosis is associated with an improved outcome</td>
<td>126</td>
</tr>
<tr>
<td>Motzkus-Feagans</td>
<td>2012</td>
<td>Statin users are 0.78 times less likely to develop C. difficile infection in the hospital</td>
<td>128</td>
</tr>
<tr>
<td>Novack</td>
<td>2012</td>
<td>Modest reduction of pneumonia incidence</td>
<td>127</td>
</tr>
<tr>
<td>Nseir</td>
<td>2012</td>
<td>Statins reduce the 30-day in-hospital all-cause mortality of bacteraemic patients</td>
<td>124</td>
</tr>
<tr>
<td>Hennessy</td>
<td>2013</td>
<td>Statins inhibit in vitro virulence phenotypes of P. aeruginosa</td>
<td>103</td>
</tr>
<tr>
<td>Doshi</td>
<td>2013</td>
<td>Survival benefit for patients receiving statins at admission for pneumococcal pneumonia</td>
<td>125</td>
</tr>
<tr>
<td>Subramanian</td>
<td>2013</td>
<td>Atorvastatin reduces periodontal inflammation</td>
<td>140</td>
</tr>
<tr>
<td>Panhar</td>
<td>2013</td>
<td>Statin reduces TB infection in macrophages and in mice</td>
<td>113</td>
</tr>
</tbody>
</table>

LPS, lipopolysaccharide; TB, Mycobacterium tuberculosis.
and endothelium, further reducing neutrophil influx.\textsuperscript{149} Indeed, in a trial of 47 CAD patients, statin reduced the concentration of soluble P-selectin, a vascular adhesion molecule participating in leucocyte adhesion to endothelial cells.\textsuperscript{150} This reduction correlated directly with the lowering of TC ($P < 0.005$) and inversely with the progression of CAD ($P < 0.0001$). In concordance, it has been recently shown that simvastatin effectively prevents neutrophil ROS production and reduces recruitment and activation of neutrophils, which limits their infiltration in vessels.\textsuperscript{47}

Diminished inflammatory cytokine release reduces inflammatory response, consequently improving endothelial function. In fact, the study of biomarkers of inflammation, oxidation, and thrombosis in samples from statin-treated patients shows reduced thrombogenesis, smooth muscle proliferation in tunica media, and matrix metalloproteinase synthesis that can bring about the observed plaque stabilization.\textsuperscript{151,152} (Figure 3).

Since it is the inflammatory response that destabilizes the atheroma, its suppression by statins reduces the plaque vulnerability independently of lowering the LDL-C, as suggested by JUPITER results.\textsuperscript{50} Reduction of plaque vulnerability is a central target of investigations, since it is plaque instability rather than lumen occlusion that may lead to acute ischaemic events.\textsuperscript{153} For details on suggested mechanisms of antimicrobial activity of statins, see Table 3.

5. Discussion

From the evidence presented above, it follows that statins may be applicable to reduce both hyperlipidaemia and all-cause mortality, including CVD-unrelated mortality. The available meta-analyses support this notion. In particular, a meta-analysis of randomized trials and cohort studies for the association between use of statins and the outcome of infections (bacteraemia ($n = 3$ studies), pneumonia ($n = 3$), sepsis ($n = 2$), and bacterial infection ($n = 1$)) favoured statin treatment. The pooled adjusted effect estimate was 0.55 (95% CI 0.36–0.83).\textsuperscript{154} In a follow-up meta-analysis of the association between preoperative statin administration and postoperative infectious complications in six cohorts of cardiac surgery patients, statin use was associated with a trend toward reduction in the incidence of postoperative infections (OR 0.81 [95% CI 0.64–1.01]).\textsuperscript{155} Lastly, a meta-analysis of the role of statins in prevention and treatment of CAP estimated a lower risk of CAP, 0.84 (95% CI 0.74–0.95) and a lower short-term mortality in patients with CAP, 0.68 (95% CI 0.59–0.78).\textsuperscript{156}

However, the evidence comes predominantly from observational studies. Also, the statin-prescribed patient cohorts may have had an advantage due to concomitant administration of anti-hypertension and other drugs, a non-specific 'health-conscious individual' phenomenon. Such medical attention bias could have been responsible for the advantages of statin treatment that have been lacking in the not so well-treated cohort. Additional prudence is dictated by the risk of concealed confounding, always present with a multifactorial condition such as CVD. Another layer of caution is due to the fact that, to reduce cholesterol synthesis, statins decrease the level of mevalonate, which is also critical for ubiquinone synthesis. Ubiquinone has antioxidant activity and is part of the mitochondrial respiration; therefore, its reduction in plasma could contribute to adverse effects such as organ failure in sepsis.\textsuperscript{157}

To underline the need of caution, the results of a multicentre, prospective cohort study of 1836 patients hospitalized with CAP were not confirmatory. Since sepsis is a leading cause of acute kidney injury and with animal studies, suggesting that the pleiotropic effect of statins attenuates the risk for acute kidney injury and decreases mortality, statin use was investigated. Of patients with acute kidney injury ($n = 631$), statin use was not associated with a significantly lower risk for death when adjusted, suggesting much caution in using statins as anti-infectives.\textsuperscript{158}

Similarly, in the case of chronic obstructive pulmonary disease (COPD), the current literature collectively suggests that statins may have a beneficial role in the treatment of COPD. However, the limitations of the majority of studies require specific trial design to assess the impact of statins on clinically relevant outcomes in COPD.\textsuperscript{159}

While high doses of statins can achieving more aggressive treatment goals, it can lead to severe necrotizing myopathy, muscle pain or weakness, myopathy, rhabdomyolysis, and myoglobinuria.\textsuperscript{160} In an important development, newer hepatoselective statins preferentially act on hepatocytes to avoid adverse effects on myocytes.\textsuperscript{161–163} Whether the resulting diminished plasma concentrations with this new class of HMG-CoA reductase inhibitors would lead to less pronounced pleiotropic effects is still not known (no data available as of February 2014).

With respect to the effect of statins on mortality in patients with infection and/or sepsis, a systematic review and meta-analysis of 20 published studies revealed the pooled ORs all in favour of statin use vs. non-use of 0.33–0.63 for 30-day mortality, specifically in-hospital mortality and pneumonia, bacteraemia, sepsis-, and mixed infection-related mortalities.\textsuperscript{164} This meta-analysis demonstrated a protective effect for statins in patients with sepsis and/or other infections; however, the results were limited by the cohort design and the degree of heterogeneity, and therefore further randomized trials are needed to validate the use of statins for sepsis and/or other infections. Consequently, since the results are variable and do not always reach statistical significance, the jury is still out on the antimicrobial effects of statins.

5.1 Clinical relevance of the mitigation of bacterial infections in addition to the lipid-lowering and the anti-inflammatory effects of statins

Only a randomized, fully controlled for lipid-lowering and anti-inflammatory effects of statins clinical trial, designed to test the
hypothesis that statins exert antimicrobial activity, could provide evidence that the proposed mitigation of bacterial infections has a clinical relevance.

So far, the results from JUPITER prospectively demonstrated that statins reduce MI, stroke, and all-cause mortality in primary prevention among healthy individuals with low LDL-cholesterol levels and elevated hsCRP.\(^50\) The JUPITER trial has not been specifically designed to examine the efficacy of anti-inflammatory activity for prevention of acute ischaemic events. This task is currently being carried out by two follow-up trials, cardiovascular inflammation reduction trial (CIRT)\(^{165}\) and CANTOS.\(^{166}\) CIRT will test the hypothesis that an anti-inflammatory (but not lipid-lowering) agent, very low-dose-methotrexate, might reduce recurrent vascular event rates and CANTOS will evaluate whether anti-inflammation cytokine-based (interleukin-1β) inhibition therapy compared with placebo will prevent secondary events (recurrent MI, stroke, and cardiovascular death) among stable patients with elevated hsCRP.\(^{167}\) In case these proof-of-concept trials turn positive, then a carefully controlled trial testing antimicrobial component of statin activity as possible component of anti-inflammatory properties may become conceivable and justified.

### 6. Conclusion

AS, the inflammatory condition underlying CVDs, is still in a need for efficient treatment modality. Despite considerable advances in drug development, the limitations of the most widely applied pharmaceutical agents—statins—and their mechanism of action remain under investigation. Meanwhile, the observed pleiotropic effects of these therapeutics, in particular the reduction of morbidity and mortality due to their anti-inflammatory and possible antimicrobial activities, seem attractive target of intensive investigation, especially in view of their relatively low cytoxicity. However, in order to (re)introduce them as a new class of antimicrobials, and at the same time as compounds modifying critical stages of atherogenesis, we would need to develop proper diagnostic modalities and conduct large-scale prospective clinical trials that would answer the following questions:

- Are there chronic inflammatory foci in this particular patient?
- What is the causative agent(s) of the infection?
- Is the infection low grade, or acute?

Accordingly, what treatment approaches are to be taken?
- How is the diagnosis going to be linked to the treatment?
- Is long-term antibiotic therapy avoidable?
- Should the treatment lead to plaque regression, or plaque stabilization is enough?

The mechanism of action of statins should be investigated at as many angles as possible specifically addressing key features of the atherosclerotic plaque—such as endothelial activation, macrophage adhesion and diapedesis, development of necrotic core (and efferocytosis, accumulation of apoptotic macrophages), smooth muscle cell proliferation, and plaque destabilization. Undoubtedly, the ability of statins to exert benefits, including at sites of infection, will attract more attention in the years to come, due to (i) the lure of having an established class of compounds as a feasible cardioprotective measure to counter the leading killer of mankind and (ii) the “patent cliff” that is now being experienced in pharmaceutical industry. The recognition that the estimated average number of years of life lost because of a MI is 16.6 (NCHS, NHBLI tabulation\(^{168}\)) is the best incentive to further explore the statins’ anti-inflammatory and antimicrobial properties.

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