Pierce et al. Scr Med 2022 Sep;53(3):247-53. DOI:10.5937/scriptamed53-37418
REVIEW ARTICLE

The Evidence for a Role of Bacteria and Viruses in Cardiovascular Disease

Grant N Pierce,[1, 2] Justin F Deniset,[3] Craig T Resch,[1, 2] Muntahi Mourin,[1, 2] Elena Dibrov,[1, 2] Pavel Dibrov[4]

Abstract

Inflammation plays a critical role in atherosclerosis and cardiovascular disease. Bacteria and viruses are major causative agents of inflammation in the body which normally develops as a response to infection. It is a logical extention, therefore, to believe bacterial and viral infections may be involved in a variety of presentations of cardiovascular diseases. The purpose of this review is to describe the data and conclusions to date on the involvement of these infectious agents in the induction of cardiovascular disease. The review also discusses the various specific bacteria and viruses that have been implicated in cardiovascular disease and the mechanisms, if known, that these agents induce cardiovascular disease.

1. Institute of Cardiovascular Sciences, Albrechtsen Research Centre, St. Boniface Hospital, Winnipeg, Manitoba R2H 2A6, Canada.
2. Department of Physiology and Pathophysiology, Rady Faculty of Health Sciences; University of Manitoba; Manitoba, Canada.
3. Department of Cardiac Sciences, Libin Institute and Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.
4. Department of Microbiology, Faculty of Science; University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada.

Correspondence:
GRANT N PIERCE
E: gpierce@sbrc.ca

ARTICLE INFO
Received: 13 April 2022
Revision received: 26 July 2022
Accepted: 26 July 2022

Infections Associated with Cardiovascular Disease

A number of risk factors have been identified as important in determining the expression of cardiovascular disease (CVD). Some are modifiable and others like genetic predisposition, time and race are not. Among the more recent environmental factors that are modifiable is infection. Over time, it has become dogma in the cardiovascular research field that infection and its associated inflammatory reactions are principle factors in the development of many CVDs. The primary type of CVD in which this presents itself is atherosclerotic CVD. The hypothesis that infection and inflammation plays a critical role in atherosclerosis created an initial surge of research to identify the specific infectious agents that may cause atherosclerotic disease. The results from these studies were somewhat surprising and disbelieved for many years. One concern for the studies was that these causative infectious agents identified were from very unlikely sources. Bacteria and viruses commonly associated with diseases of non-cardiac origin were implicated in CVD. However, the evidence to strongly support their involvement has accumulated over many years and now even includes the virus involved in the

This article should be cited as follows: Pierce GN, Deniset JF, Resch CT, Mourin M, Dibrov E, Dibrov P. The evidence for a role of bacteria and viruses in cardiovascular disease. Scr Med 2022 Sep;53(3):247-53.

most recent COVID-19 pandemic. The purpose of this review is to discuss the various bacteria and viruses that have been implicated in CVD and the mechanisms, if known, that these agents induce CVD.

Bacterial Infections Associated with Cardiovascular Disease

A variety of bacteria, commonly associated with diseases of non-cardiac origin, have nonetheless been implicated in CVD. All are Gram-negative bacteria. For example, although *Helicobacter pylori* is most commonly associated with persistent gastritis,[1] these bacteria have also been identified as causative agents in CVD. *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* are causative bacteria for periodontal disease but have also been implicated in CVD. *Chlamydia pneumoniae* and *Mycobacterium tuberculosis* are well known for their respiratory complications,[2] however, these bacteria have also been identified as causative agents in CVDs.

Chlamydia pneumoniae is the most intensely studied pathogen with regard to its role in CVD. *C pneumoniae* antibodies have been detected within the serum of patients with coronary artery disease.[3, 4] DNA and elementary bodies of *C pneumoniae* have also been found in the atherosclerotic plaque.[2] Most importantly, a causal relationship and the mechanisms of action for *C pneumoniae* infection and atherogenesis has been tested and established in some cases,[5-9] although some aspects remain in dispute.[10, 11] Heat inactivated *C pneumoniae* is not atherogenic.[12]

H pylori has been strongly correlated with atherosclerotic heart disease and coronary artery disease and stroke clinically,[13-15] particularly in patients with hyperhomocysteinaemia.[16] Causal evidence for a role for *H pylori* in atherogenesis and coronary artery disease is lacking.

P gingivalis is one of several bacterial pathogens commonly associated with dental plaque and gum disease. This includes *Actinobaccillus actinomycetemcomitans, Treponema denticola* and *Bacteroides forsythus*. Again, there is strong evidence of a close correlation of *P gingivitis* with coronary artery disease with DNA evidence of its presence in up to 30 % of carotid atheromas.[17, 18] Furthermore, CVD risk has been correlated with the severity of the periodontal infection.[19]

The Mechanisms Whereby Bacterial Infections Induce Cardiovascular Disease

Further studies have clearly demonstrated that the relationship between infection and atherosclerosis is not a simple indirect association but an important cause and effect relationship.[5-7, 20-25] The mechanism whereby infections induce an atherogenic lesion is unclear. Many of these infectious agents either have stimulatory effects on cellular proliferation[8, 9, 23, 26, 27] or inhibitory effects on apoptosis.[28] These actions may be involved in their atherogenic effects.[23, 26, 28] The susceptibility of atherogenesis to such a broad spectrum of infectious agents, however, suggests that a pathway common to infection in general may be a key player in this phenomenon. Heat shock proteins have been identified as one potential mediator that would link the atherosclerosis process with the infection/inflammatory condition.[28-32] It is also clear that lipids have an important role to play in modifying the atherogenic effects of infections.[5, 9, 33] It has been identified that the atherogenic potential of *C pneumoniae* is dependent upon a high cholesterol environment.[5, 7] Feeding the LDL receptor knockout mouse a high cholesterol diet induced atherosclerotic plaque formation. Conversely, infection of mice on a normal diet with *C pneumoniae* did not induce an atherosclerotic effect. However, if the animals were placed on the cholesterol-enriched diet to induce atherosclerosis and then infected with *C pneumoniae*, a significantly greater stimulation of atherosclerotic plaque formation[5, 7] than cholesterol could induce on its own was observed. These data strongly suggested a synergistic interaction between the infection and cholesterol. This model is attractive because it links an important conventional atherogenic risk factor (cholesterol) with the infection process and may explain why some people develop clinically significant atherosclerosis after an infection and others do not. Relevant to the present topic, it also strongly points to the importance of diet in modulating the atherogenic action of an infectious agent. Significantly, a

clinical investigation has documented the potent stimulatory effect of additional risk factors like hypercholesterolaemia on the cardiovascular effects of infection with *C pneumoniae* in a clinically relevant patient population.[34]

Antibiotic Use to Prevent CVD

If these bacteria do play a role in atherosclerotic CVD, the next logical step would be to determine if antibiotic usage can prevent or deter the clinical symptoms of CVD. *C pneumoniae* strains are susceptible to antibiotics like tetracycline, fluoroquinolones and macrolides in *in vitro* studies.[35, 36] However, the results from clinical trials testing the capacity of antibiotics to prevent major CVD events have been disappointing. Retrospective studies in patients treated with antibiotics prior to a myocardial infarction have yielded no clear conclusions.[37, 38] A detailed description of the clinical trials using antibiotics to prevent CVD is found elsewhere.[39] In summary, although the first few small antibiotic trials with azithromycin on patients with cardiovascular disease resulted in a positive protective effect versus the incidence of myocardial infarctions and indices of CVD,[40-43] several more extensive trials like the ACADEMIC study (The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia),[44] the WIZARD trial (Weekly Intervention With Azithromax Against Atherosclerotic-Related Disorders),[45] the CLARICOR trial (CLARIthromycin for patients with stable CORonary heart disease),[46] the ACES trial (Azithromycin and Coronary Events Study),[47] the ANTIBIO trial (Antibiotic Therapy in Acute Myocardial Infarction),[48] the AZACS trial (Azythromycin in Acute Coronary Syndrome)[49] and the PROVE-IT trial (Pravastatin Or Atorvastatin Evaluation and Infection Therapy)[50] all concluded that antibiotic treatments did not result in any reduction in cardiovascular events. As a result, the use of antibiotics to prevent CVD have been largely abandoned as a viable approach. The reason for the lack of prevention may be multifactorial. It is clear that macrolides do not impact persistent *Chlamydial* infection during its persistent stage[39] and this persistence of bacterial infection would negate any antibiotic effect of the macrolides tested. Murine models of atherosclerosis have identified a high cholesterol environment as being critical to the atherogenic effects of *C pneumoniae* infection.[5] It is possible, therefore, that antibiotic treatment may be best utilised in a clinical population with high circulating cholesterol levels prior to the appearance of clinically significant cardiovascular disease. Some clinical studies would lend support to this hypothesis[34] but further work is required to substantiate this conclusively. Additionally, it is possible that a critical window of time exists for the preventative effect of the antibiotics. Antibiotics delivered to rabbits shortly after bacterial inoculation inhibited *C pneumoniae*-induced atherosclerosis but was ineffective if delivered 6 weeks post-inoculation.[51] Clinical trials using antibiotics were delivered in patients with well established cardiovascular disease. The alternative approach of testing the delivery of antibiotics chronically in order to prevent the appearance of atherosclerotic plaque formation before it is established has been proposed but never tested. Today, with the ever growing emergence of multidrug resistance in pathogenic bacteria as a serious health problem, an approach of using antibiotics over an extended period of time when there is an absence of clinical evidence of an infection would not be feasible. This strategy for the prevention of cardiovascular disease would undoubtedly promote the acceleration of antibiotic resistance in pathogenic bacteria and this would be an unacceptable consequence.

Other approaches besides antibiotics for the treatment of infection-induced atherosclerotic disease could be advanced. The afore-mentioned relationship of cholesterol with *C pneumoniae*-induced infection may offer intriguing options. An oxidation mechanism has been proposed to be involved in the association of cholesterol with *C pneumoniae*-induced atherogenesis. It is well known that *C pneumoniae* can induce a stimulation of intracellular oxidative processes.[52, 53] For example, *C pneumoniae* and other infectious processes can induce oxidation of LDL in *in vitro* and *in vivo* settings.[52-56] Oxidized LDL is thought to play an important role in atherosclerosis.[57-59] Oxidized LDL can potentiate the mitogenic actions of *C pneumoniae*.[9] It is possible, therefore, that the oxidation induced by infectious agents like *Chlamydia pneumoniae*[9] is critical to the atherogenic event and preventing this process may retard or block the atherogenic action of *Chlamydia* and other infectious organisms. Blocking the oxidation by treating the *C pneumoniae*-induced cardiovascular disease with

antioxidants has not been tested but may provide positive results.

Other alternative pathways have been identified as associated with the atherogenic actions of infectious stimuli like *C pneumoniae*. It has been suggested[39] for example, that it may be more effective to target downstream pathways induced by the *Chlamydial* infection rather than the bacteria itself. Targeting the inflammatory and mitogenic pathways that are activated by the infection to generate the atherogenesis may be more beneficial, particularly those involving heat shock protein 60,[27, 29] specific pattern recognition receptors, transporters or other target proteins.[39]

Viral Infections Associated with Cardiovascular Disease

Bacteria are not the only infectious agents that have been associated with cardiovascular diseases. Herpes simplex virus (HSV),[23, 24] Epstein-Barr virus DNA and cytomegalovirus (CMV),[28, 60] have been tied to both atherosclerotic cardiovascular and cerebrovascular lesions. More recently, another viral infection, the Coronavirus 2019 (COVID-19), has also been strongly linked to a variety of cardiovascular diseases by a host of new studies.

Siscovick and colleagues found that herpes simplex virus type 1 (HSV) IgG antibodies were associated with a 2 fold increase in the risk of incident myocardial infarction and coronary heart disease in older adults.[61] Another study discovered elevated levels of HSV, CMV and Epstein-Barr virus DNA in coronary artery atherosclerotic plaques obtained by end-arterectomy.[62] A large investigation, the ARIC study (Atherosclerosis Risk in Communities), reported that high levels of CMV antibodies were significantly associated with incident coronary heart disease.[63] In another large study of over 14,000 patients, CMV was associated with a significant increase in the risk for all-cause mortality and, when combined with elevated CRP levels, the risk for all-cause mortality and CVD-associated mortality also increased.[64] Serum antibody levels to HSV and two periodontal infections were measured in over 1100 Finnish and Russian participants.[65] They found the risk for CVD was increased when the antibody levels for the three infectious agents were elevated. These studies have led to the hypothesis that the entire burden of simultaneous chronic infectious disease may be the most important factor in the induction of CVD.[66]

Conflicting evidence arguing against a role for HSV and CMV in CVD has been reported. The prevalence of CMV and HSV in coronary artery disease patients was not elevated.[67] Siscovick et al[61] did not find that an elevation in IgG antibodies to CMV was associated with CVD risk among the elderly. Ridker et al[68] also found no correlation between HSV or CMV IgG antibody titers and subsequent risk for CVD in postmenopausal women. More negative results have been reported for CMV in another recent study.[69] The conflicting evidence, particularly for CMV, would lead one to conclude that there is a need for further large controlled studies to resolve this situation.

The emergence of the COVID-19 pandemic has resulted in the evolution of strong evidence for another association between an infectious agent and CVD. Indeed, it may be argued that the controversy that has arisen in the field as to the involvment of viruses in CVD has been answered unquestionably by the clear association of the COVID-19 virus with both acute and chronic manifestations of CVD. The COVID-19 zoonotic viral protein acts initially by binding to the angiotensin converting enzyme 2 (ACE2) receptor to ultimately gain entry to the cell.[70] The strong evidence for a role for the ACE2 receptors in the CVD induced by COVID-19 infection should not come as too big a surprise. ACE2 receptors have long been associated with CVD indiependently of COVID-19 infection.[71-73] This includes the overexpression of ACE2 activity in failing hearts.[73] The resultant systemic inflammation caused by COVID-19 infection induces an intense immune and cytokine response followed by plaque destabilisation, acute coronary syndrome, hypoxia, myocarditis, arrhythmias, cardiomyopathy, cardiogenic shock, myocardial damage, cardiac arrest and ultimately heart failure.[74] Venous thromboembolism and platelet aggregation have also been observed in COVID-19-infected patients.[70]

Some 20-30 % of patients admitted to hospitals with COVID-19 infections have cardiovascular complications.[75] Pre-existing CVD appears to exacerbate the effects of COVID-19 infection.[75] Even in patients who had recovered from COVID-19 infection, magnetic resonance imaging detected myocardial damage and myocardial inflammation in 78 % and 60 %, respectively, of these patients.[76, 77] The cardiovascular

pathologies may be linked to an increased risk of venous and arterial thromboembolic events.[77] Arrhythmias persist post COVID-19 recovery and the emergence of cardiomyopthies subsequent to infection have also been reported.[75] This further supports the contention that COVID-19 infection is associated with a persistent and chronic CVD.[75] Futhermore, the strikingly higher male mortality incidence in COVID-19 infected patients may be explained by the sexual dimorphism in pre-existing cardiovascular comorbidities.[78]

Conclusion

The majority of evidence over decades of investigation supports the involvement of both bacterial and viral infection as having an important role to play in inducing CVD. It is also possible now to come to a tentative conclusion that different bacteria and different viruses have very different capacities to induce CVD and, furthermore, they will induce varying severities of CVD. Perhaps the most dramatic example of a viral infection being associated with CVD is the recent pandemic with COVID-19. The infection has been associated with both an immediate as well as a chronic manifestation of a panapoly of CVDs. This is likely due to its uniquely facile access to the cardiovascular tissue through the ACE-2 receptor. Unfortunately, the serious vascular and cardiac effects of the current COVID-19 pandemic may only be a harbinger of the future challenges facing the cardiovascular system when multidrug resistance to antibiotics escalates even further and unleashes a horde of pathogenic bacteria on a defenceless cardiovascular system.[79]

Acknowledgements

This work was supported by a Foundation Grant from CIHR to GNP, and an NSERC grant to PD.

Conflict of interest

None.

References

1. Watari J, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, et al. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J Gastroenterol 2014 May 14;20(18):5461-73.
2. Cheung PKM, Pierce GN. The role of infectious agents in atherogenesis. In: Cheema SK, editor. Biochemistry of atherosclerosis. New York (USA): Springer Publishing; 2006. p. 413-434.
3. Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988;2(8618):983-6.
4. Dahlen GN, Boman J, Birgander LS, Lindblom B. Lp(a) lipoprotein, IgG, IgA and IgM antibodies to Chlamydia pneumoniae and HLA class II hgenotype in early coronary artery disease. Atheroclerosis 1995;114:165-74.
5. Hu H, Pierce GN, Zhong G. The atherogenic effects of Chlamydia are dependent upon serum cholesterol and specific to the Chlamydia pneumoniae species. J Clin Invest 1999;103:747-53.
6. Fong IW, Chiu B, Viira E, Jang D, Mahony JB. De novo induction of atherosclerosis by Chlamydia pneumoniae in a rabbit model. Infect Immun 1999;67:6048-55.
7. Liu L, Hu H, Ji H, Murdin AD, Pierce GN, Zhong G. Chlamydia pneumonia infection significantly exacerbates aortic atherosclerosis in an LDLR-/- mouse model within six months. Mol Cell Biochem 2000;215:123-8.
8. Deniset JF, Cheung PKM, Dibrov E, Lee K, Steigerwald S, Pierce GN. Chlamydophila pneumoniae infection leads to smooth muscle cell proliferation and thickening in the coronary artery without contributions from a host immune response. Am J Pathol 2010;176:1028-37.
9. Chahine MN, Deniset J, Dibrov E, Hirono S, Blackwood DP, Austria JA, et al. Oxidized LDL promotes the mitogenic actions of Chlamydia pneumoniae in vascular smooth muscle cells. Cardiovasc Res 2011;92:476-83.
10. Caligiuri G, Rottenberg M, Nicoletti A, Wigzell H and Hansson GK. Chlamydia pneumoniae infection does not induce or modify atherosclerosis in mice. Circulation 2001;103(23):2834-8.
11. Aalto-Setala K, Laitinen K, Erkkila L, Leinonen M, Jauhiainen M, Ehnholm C, et al. Chlamydia pneumoniae does not increase atherosclerosis in the aortic root of apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2001;21:578-84.
12. Sharma J, Niu Y, Ge J, Pierce GN and Zhong G. Heat-inactivated C pneumoniae organisms are not atherogenic. Mol Cell Biochem 2004;260:147-52.
13. Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, et al. Relation of Helicobacter pylori infection and coronary heart disease. Br Heart J 1994;71:437-9.
14. Strachan DP, Mendall MA, Carrington D, Butland BK, Yarnell JW, Sweetnam PM, et al. Relation of Helicobacter pylori infection to 13-year mortality and incident ischemic heart disease in the Caerphilly prospective heart disease study. Circulation 1998:98:1286-90.
15. Sawayama Y, Ariyama I, Hamada M, Otaguro S, Machi T, Taira Y, et al. Association between chronic Helicobacter pylori infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis 2005;178(2):303-9.
16. Sung JJ, Sanderson JE. Hyperhomocysteinaemia, He-

licobacter pylori and coronary heart disease. Heart 1996;76(4):305-7.
17. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71(10):1554-60.
18. Chiu B. Multiple infections in carotid atherosclerotic plaques. Am Heart J 1999;138:S534-S536.
19. Armitage GC. Periodontal infections and cardiovascular disease – how strong is the association? Oral Dis 2000;6:335-50.
20. Saikku P. Chlamydia pneumoniae in atherosclerosis. J Intern Med 2000;247:391-6.
21. Muhlestein, JB. Chlamydia pneumoniae-induced atherosclerosis in a rabbit model. J Infect Dis 2000;181(Suppl 3): S505-7.
22. Campbell, LA, Blessing E, Rosenfeld M, Lin TM, Kuo C. Mouse models of C pneumoniae infection and atherosclerosis. J Infect Dis 2000;181(Suppl 3):S508-13.
23. Miyatake SI, Yukawa H, Toda H, Matsuoka N, Takahashi R, Hashimoto N. Inhibition of rat vascular smooth muscle cell proliferation in vitro and in vivo by recombinant replication-Competent herpes simplex virus. Stroke 1999;30: 2431-9.
24. Lin TM, Jiang MG, Eng HL, Shi GY, Lai LC, Huang BJ, et al. Experimental infection with bovine herpesvirus-4 enhances atherosclerotic process in rabbits. Lab Invest 2000;80(1):3-11.
25. Moazed TC, Campbell LA, Rosenfeld ME, Grayston JT, Kuo CC. Chlamydia pneumoniae infection accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. J Infect Dis 1999;180:238-41.
26. Zhou, YF, Yu ZX, Wanishsawad C, Shou M, Epstein SE. The immediate early gene products of human cytomegalovirus increase vascular smooth muscle cell migration, proliferation, and expression of PDGF beta-receptor. Biochem Biophys Res Commun 1999;1999;256: 608-13.
27. Hirono S, Dibrov E, Hurtado C, Kostenuk A, Ducas R, Pierce GN. Chlamydia pneumoniae stimulates proliferation of vascular smooth muscle cells through induction of endogenous heat shock protein 60. Circ Res 2003;93:710-6.
28. Tanaka K, Zou JP, Takeda K, Ferrans VJ, Sandford GR, Johnson TM, et al. Effects of human cytomegalovirus immediate-early proteins on p53-mediated apoptosis in coronary artery smooth muscle cells. Circulation 1999;99: 1656-9.
29. Deniset JF, Pierce GN. Heat shock proteins: Mediators of atherosclerotic development. Current Drug Targets 2015;16:816-26.
30. George J, Shoenfeld Y, Gilburd B, Afek A, Shaish A, Harats D. Requisite role for interleukin-4 in the acceleration of fatty streaks induced by heat shock protein 65 or Mycobacterium tuberculosis. Circ Res 2000;86:1203-10.
31. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: Heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol Res 2000;164:13-7.
32. Xu Q, Detrich H, Steiner HJ, Gown AM, Schoel B, Mikuz G, et al. Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. Arterioscler Thromb 1992;12:789-99.
33. Laurila A, Bloigu A, Nayha S, Hassi J, Leinonen M, Saikku P. Association of Helicobacter pylori infection with elevated serum lipids. Atherosclerosis 1999;142: 207-10.
34. Kinjo K, Sato H, Sato H, Ohnishi Y, Hishida E, Nakatani D, et al. Joint effects of Chlamydia pneumonia infection and classic risk factors on risk of acute myocardial infarction. Am Heart J 2003;146:324-30.
35. Chirgwin K, Roblin PM, Hammerschlag MR. In vitro susceptibilities of Chlamydia pneumoniae (Chlamydia sp. strain TWAR)Antimicrob Agents Chemother 198933:1634-5.
36. Gieffers J, Solbach W, Maass M. In vitro susceptibilities of Chlamydia pneumoniae strains recovered from atheroscleotic coronary arteries. Antimicrob Agents Chemother 1998;42:2762-64.
37. Jackson LA, Smith NL, Heckbert SR, Grayston, JT, Siscovick DS, Psaty BM. Lack of association between first myocardial infarction and past use of erythromycin, tetracycline or doxycycline. Emerg Infect Dis 1999;5:281-4.
38. Meier CR, Derby LE, Jick SS, Vasilakis C, Jick H. Antibiotics and risk of subsequent first-time acute myocardial infarction. JAMA 1999;281:427-31.
39. Deniset JF, Pierce GN. Possibilities for therapeutic interventions in disrupting Chlamydophila pneumonia involvement in atherosclerosis. Fund Clin Pharmacol 2010;24:607-17.
40. Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ. Elevated Chlamydia pneumoniae antibiotics, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Ciculation 1997;96:404-7.
41. Gurfinkel E, Bozovich G, Beck E, Testa E, Livellara B, Mautner B. Treatment with the antibiotic roxithromycin I patients with actue non-Q-wave coronary syndromes. The final report of the ROXIS Study. Eur Heart J 1999;20;121-7.
42. Stone AF, Mendall MA, Kaski JC, Edger TM, Risley P, Poloniecki J, et al. Effect of treatment for Chlamydia pneumoniae and Helicobacter pylori on markers of inflammation and cardiac events in patients with actue coronary syndromes: South Thames Trial of Antibiotics in Myocardial Infaraction and Unstable Angina (STAMINA). Circulation 2002;106;1219-23.
43. Sinisalo J, Mattila K, Valtonen V, Anttonen O, Juvonen J, Merlin J, et al. Effect of 3 months of antimicrobial treatment with clarithromycin in actue non-q-wave coronary syndrome. Circulation 2002;105;1555-60.
44. Muhlestein JB, Anderson JL, Carlquist JF, Salunkhe K, Horne BD, Pearson, RR, et al. Randomized secondary prevention trial of azithromycin in patients with coronary artery disease: primary clinical results of the ACADEMIC study. Circulation 2000;102;1755-60.
45. O'Connor CM, Dunne MW, Pfeffer MA, Muhlestin JB, Yao L, Gupta S, et al. Azithromycin for the secondary prevention of coronary heart disease events: the WIZARD study: a randomized controlled trial. JAMA 2003;290;1459-66.
46. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helo OH, et al. Randomized placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ 2006;332;22-7.
47. Grayston JT, Kronmal RA, Jacson LA, Parisi AF, Muhlestein JB, Cohen JD, et al. Azithromycin for the secondary prevention of coronary events. N Engl J Med 2005;352:1637-45.
48. Zahn R, Schneider S, Friling B, Seidl K, Tebbe U, Weber M, et al. Antibiotic therapy after actue myocardial infarction:a prospective randomized study. Circulation 2003;107:1253-9.
49. Cercek B, Shah PK, Noc M, Zaher D, Zeymer U, Matetzky S, et al. Effect of short-term treatment with azithromycin n recurrent ischaeic events in patients with

actue coronary syndrome in the Azithromycin in Acute Coronary Syndrome (AZACSS) trial: a randomised controlled trial. Lancet 2003;361:809-13.
50. Cannon CP, Braundwald E, McCabe CH, Grayston JT, Muhlestein B, Giugliano RP, et al. Antibiotic treatment of Chlamydia pneumoniae after actue coronary syndrome. N Engl J Med 2005;352:1646-54.
51. Fong IW. Value of animal models for Chlamydia pneumoniae-related atherosclerosis. Am Heart J 1999;138:S512-513.
52. Kalayoglu MV, Hoerneman B, LaVerda D, Morrison SG, Morrison RP, Byrne GI. Cellular oxidation of low-density lipoprotein by Chlamydia pneumoniae. J Infect Dis 1999;180:780-90.
53. Tosi MF, Hammerschlag MR. Chlamydia trachomatis selectively stimulates myeloperoxidase release but not superoxide production by human neutrophils. J Infect Dis 1988;158:457-60.
54. Khovidhunkit W, Memon RA, Feingold KR, Grunfeld C. Infection and inflammation-induced proatherogenic changes of lipoproteins. J Infect Dis 2000;181(Suppl 3):S462-72.
55. Kalayoglu M, Miranpuri GS, Golenbock DT, Byrne GI. Characterization of low-density lipoprotein uptake by murine macrophages exposed to Chlamydia pneumoniae. Microbes Infect 1999;1:409-18.
56. Memon RA, Noor ISM, Holleran WM, Uchida Y, Moser AH, Feingold KR, et al. Infection and inflammation induce LDL oxidation in vivo. Arterioscler Thromb Vasc Biol 2000;20:1536-42.
57. Reaven PD, Witztum JL. Oxidized low density lipoproteins in atherogenesis: Role of dietary modification. Annu Rev Nutr 1996;16:51-71.
58. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991;88:1785-92.
59. Berliner JA, Navab M, Fgelman AM, Frank JS, Demer LL, Edwards PA, et al. Atherosclerosis: Basic mechanisms oxidation, inflammation, and genetics. Circulation 1995;91:2488-96.
60. Wanishsawad C, Zhou YF, Epstein SE. Chlamydia pneumoniae-Induced transactivation of the major immediate early promoter of cytomegalavirus: Potential synergy of infectious agents in the pathogenesis of atherosclerosis. J Infect Dis 2000;181:787-90.
61. Siscovick DS, Schwartz SM, Corey L, Grayston JT, Ashley R, Wang SP, et al. Chlamydia pneumoniae, herpex simplex virus type 1 and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults: the Cardiovascular Health Study. Circulation 2000;Nov 7;102(19):2335-40.
62. Ibrahim AI, Obeid M, Jouma MJ, Moasis GA, Al-Richane WL, Kindermann I, et al. Detection of herpes simplex virus, cytomegalovirus and Epstein-Barr virus DNA in atherosclerotic plaques in unaffected bypass grafts. J Clin Virol 2005; Jan;32(1):29-32.
63. Sorlie PD, Nieto FJ, Adam E, Folsom AR, Shahar E, Massing M. A prospective study of cytomegalovirus, herpes simplex virus 1 and coronary heart disease: the atherosclerosis risk in communities (ARIC) study. Arch Intern Med 2000;July 10;160(13):2027-32.
64. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. PLos One 2011;Feb 17;6(2):e16103. doi: 10.1371/journal.pone.0016103.
65. Vilkuna-Rautiainen T, Pussinen, PJ, Roivainen,M, Petays T, Jousilahti P, Hovi T, et al. Serum antibody response to periodontal pathogens and herpes simplex virus in relation to classic risk factors of cardiovascular disease. Int J Epidemiol 2006;Dec;35(6):1486-94.
66. Armitage GC. Periodontal infections and cardiovascular disease-how strong is the association? Oral Dis 2000;Nov;6(6):335-50.
67. Dadashi M, Hajikhani B, Ghazi M, Yazdani S, Goudarzi M, Nasiri MJ, et al. The global prevalence of Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus and herpes simplex virus in patients with coronary artery disease: A systematic review and meta-analysis. Microb Pathog 2021;Mar;152:104572. doi: 10.1016/j.micpath.2020.104572.
68. Ridker PM, Hennekens CH, Buring JE, Kundsin R, Shih J. Baseline IgG antibody titers to Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus, and cytomegalovirus and the risk for cardiovascular disease in women. Ann Intern Med 1999;Oct(19);131(8):573-7.
69. Stockdale L, Nash S, Nalwoga A, Painter H, Asiki G, Fletcher H, et al. Human cytomegalovirus epidemiology and relatioship to tuberculosis and cardiovascular disease risk factors in a rural Ugandan cohort. PLos One 2018;Feb 6;13(2):e0192086. doi: 10.1371/journal.pone.0192086.
70. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease:from basic mechanisms to clinical perspectives. Nat Rev Cardiol 2020;Sep:17(9):543-58.
71. Hussain A, Tang O, Sun C, Jia X, Selvin E, Nambi V, et al. Soluble angiotensin-converting enzyme 2, cardiac biomarkers, structure, and function, and cardiovascular events (from the atherosclerosis risk in communities study). Am J Cardiol 2021;146:15–21.
72. Narula S, Yusuf S, Chong M, Ramasundarahettige C, Rangarajan S, Bangdiwala SI, et al. Plasma ACE2 and risk of death or cardiometabolic diseases: A case-cohort analysis. Lancet 2020;396:968–76.
73. Zisman LS, Keller RS, Weaver B, Lin Q, Speth R, Bristow MR, Canver CC. Increased angiotensin-(1-7)-forming activity in failing human heart ventricles: evidence for upregulation of the angiotensin-converting enzyme Homologue ACE2. Circulation 2003;108(14):1707-12.
74. Magadum A, Kishore R. Cardiovascular manifestations of COVID-19 infection. Cells 2020;Nov 19;9(11):2508. doi: 10.3390/cells9112508.
75. Massaeli H, Austria JA, Pierce GN. Increase in nuclear calcium in smooth muscle cells exposed to oxidized low density lipoprotein. Free Radical Res 2001;34:9-16.
76. Puntmann VO, Carerj M L, Wieter I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus diease 2019 (COVID-19). JAMA Cardiol 2020;Nov 1;5(11):1265-73.
77. Ali MAM, Spinler SA. COVID-19 and thrombosis:-from bench to bedside. Trends Cardiovasc Med 2021;31(3):143-60.
78. Bienvenu LA, Noonan J, Wang X, Peter K. Higher mortality of COVID-19 I males: sex differences in immune response and cardiovascular comorbidites. Cardiovasc Res 2020;Dec 1:116(14):2197-206.
79. Pierce GN, Resch C, Mourin M, Dibrov P, Dibrov E, Ravandi A. Bacteria and the growing threat of multidrug resistance for invasive cardiac interventions. Rev Cardiovasc Med 2022 Jan 14;23(1):15. doi: 10.31083/j.rcm2301015.