The roles of neutrophils linking periodontitis and atherosclerotic cardiovascular diseases

Rizky A. Irwandi[1], Scott T. Chiesa[2], George Hajishengallis[3], Venizelos Papayannopoulos[4], John E. Deanfield[2], Francesco D'Aiuto[1*]

[1]Eastman Dental Institute, Faculty of Medical Sciences, University College London, United Kingdom, [2]Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, United Kingdom, [3]Department of Basic and Translational Sciences, School of Dental Medicine, University of Pennsylvania, United States, [4]Laboratory of Antimicrobial Defense, Francis Crick Institute, United Kingdom

Submitted to Journal:
Frontiers in Immunology

Specialty Section:
Molecular Innate Immunity

Article type:
Review Article

Manuscript ID:
915081

Received on:
07 Apr 2022

Revised on:
29 May 2022

Journal website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

RAI wrote the original draft. RAI, STC, GH, VP, JED, and FD'A provided critical revisions to the article. All authors contributed to the article and approved the submitted version.

Keywords

Neutrophils, systemic inflammation, trained immunity, Innate immune memory, Periodontitis, Periodontal disease, Atherosclerosis, Atherosclerotic cardiovascular disease

Abstract

Word count: 180

Inflammation plays a crucial role in the onset and development of atherosclerosis. Periodontitis is a common chronic disease linked to other chronic inflammatory diseases such as atherosclerotic cardiovascular diseases (ASCVD). The mechanistic pathways underlying this association are yet to be fully understood. This critical review aimed at discussing the role of neutrophils in mediating the relationship between periodontitis and ASCVD. Systemic inflammation triggered by periodontitis could lead to adaptations in hematopoietic stem and progenitor cells (HSPCs) resulting in trained granulopoiesis in the bone marrow, thereby increasing the production of neutrophils and driving the hyper-responsiveness of these abundant innate-immune cells. These alterations may contribute to the onset, progression, and complication of atherosclerosis. Despite the emerging evidence suggesting that the treatment of periodontitis improves surrogate markers of cardiovascular disease, the resolution of periodontitis may not necessarily reverse neutrophil hyper-responsiveness since the hyper-inflammatory re-programming of granulopoiesis can persist long after the inflammatory inducers are removed. Novel and targeted approaches to manipulate neutrophil numbers and functions are warranted within the context of the treatment of periodontitis also to mitigate its potential impact on ASCVD.

Contribution to the field

Inflammation plays a crucial role in the onset and development of atherosclerosis. Periodontitis is a common chronic disease linked to other chronic inflammatory diseases such as atherosclerotic cardiovascular diseases. The mechanistic pathways underlying this association are yet to be fully understood. We discussed the role of neutrophils in mediating the relationship between periodontitis and atherosclerotic disease. Systemic inflammation triggered by periodontitis could lead to adaptations in bone marrow progenitor cells resulting in trained granulopoiesis in the bone marrow, thereby increasing the production of neutrophils and driving the hyper-responsiveness of these abundant innate-immune cells. These alterations may contribute to the onset, progression, and complications of atherosclerosis. Despite the emerging evidence suggesting that the treatment of periodontitis improves surrogate markers of cardiovascular disease, the resolution of periodontitis may not necessarily reverse neutrophil hyper-responsiveness since the hyper-inflammatory re-programming of granulopoiesis can persist long after the inflammatory inducers are removed. We then discussed the current approaches to target neutrophils in treating both atherosclerotic cardiovascular disease and periodontitis. Collectively, this review provides some molecular basis of neutrophils in linking periodontitis to atherosclerotic cardiovascular disease.

The roles of neutrophils linking periodontitis and atherosclerotic cardiovascular diseases

Rizky A. Irwandi[1], Scott T. Chiesa[2], George Hajishengallis[3], Venizelos Papayannopoulos[4], John E. Deanfield[2], Francesco D'Aiuto[1]

[1]Periodontology Unit, UCL Eastman Dental Institute, University College London, London, United Kingdom

[2]UCL Institute of Cardiovascular Science, University College London, London, United Kingdom

[3]Department of Basic & Translational Sciences, Laboratory of Innate Immunity & Inflammation, University of Pennsylvania, Penn Dental Medicine, Philadelphia USA

[4]Antimicrobial Defense Laboratory, The Francis Crick Institute, London, United Kingdom

*** Correspondence:**
Francesco D'Aiuto
f.daiuto@ucl.ac.uk

Keywords: neutrophils, systemic inflammation, trained immunity, innate immune memory, periodontitis, periodontal disease, atherosclerosis, atherosclerotic cardiovascular disease.

Abstract

Inflammation plays a crucial role in the onset and development of atherosclerosis. Periodontitis is a common chronic disease linked to other chronic inflammatory diseases such as atherosclerotic cardiovascular diseases (ASCVD). The mechanistic pathways underlying this association are yet to be fully understood. This critical review aimed at discussing the role of neutrophils in mediating the relationship between periodontitis and ASCVD. Systemic inflammation triggered by periodontitis could lead to adaptations in hematopoietic stem and progenitor cells (HSPCs) resulting in trained granulopoiesis in the bone marrow, thereby increasing the production of neutrophils and driving the hyper-responsiveness of these abundant innate-immune cells. These alterations may contribute to the onset, progression, and complication of atherosclerosis. Despite the emerging evidence suggesting that the treatment of periodontitis improves surrogate markers of cardiovascular disease, the resolution of periodontitis may not necessarily reverse neutrophil hyper-responsiveness since the hyper-inflammatory re-programming of granulopoiesis can persist long after the inflammatory inducers are removed. Novel and targeted approaches to manipulate neutrophil numbers and functions are warranted within the context of the treatment of periodontitis also to mitigate its potential impact on ASCVD.

1 Introduction

Atherosclerotic cardiovascular disease (ASCVD) consists of a group of disorders that affect heart and blood vessel (1) and include coronary heart disease, cerebrovascular disease, and peripheral vascular disease (2). ASCVD is a major cause of global mortality and leading contributor to disability as it causes 18.6 million deaths and contributes to 34.4 million people lives with disability in 2019 (3).

Although its pathogenesis, progression, and complications comprise of multiple complex processes, inflammation presents a key role in each stage of the disease (4).

Periodontitis is a common chronic inflammatory disease caused by oral microbial dysbiosis. The onset and progression of the disease could span over decades and are influenced by genetic and environmental factors. This prevalent oral disease is characterized by progressive destruction of hard and soft tissues supporting the tooth, including periodontal ligament and alveolar bone (5). Untreated periodontitis leads inevitably not only to tooth loss but also to masticatory impairment and negative influences on the patient's quality of life (6). Like ASCVD, periodontitis is a major public health concern as it affects over the half of world's population (7) and 5-15% of the global population presented a severe form of the disease (8) causing increased costs of oral healthcare (9).

The evidence linking periodontitis to systemic diseases previously focused on the findings that periodontal bacteria and their endotoxins disseminate physically through the blood circulation (10, 11). However, periodontitis also triggers systemic inflammation indicated by an increased level of C-reactive protein (CRP), TNFα, IL-1β, and IL-6 in the serum of patients (2). As a result of chronic inflammation occurring at the periodontium, endotoxemia, bacteremia, and systemic inflammation collectively are implicated in numerous systemic diseases including atherosclerotic cardiovascular disease (ASCVD) (12-14).

Neutrophils are the most abundant inflammatory cells in humans and the first-line defense against infection in innate-arm of immune system. They are derived from the myeloid differentiation lineage of hematopoietic stem cells (HSCs) in the bone marrow. Upon detection of pathogens, neutrophils capture and destroy invading pathogens via phagocytosis and intracellular degradation, degranulation, and formation of neutrophil extracellular traps (NETs) (15). Moreover, the emerging evidence over the past decade reveals that neutrophils are involved in chronic inflammation and implicated in chronic inflammatory disorders including periodontitis and ASCVD (16-20). Periodontitis appears to be associated with hyper-responsive neutrophils (21-23) which might, at least in part, be attributed to the notion that oral disease could influence hematopoietic tissue activity and trained immunity (12). Trained immunity represents a non-specific memory in innate immune cells that is induced by earlier encounters with infectious or inflammatory stimuli, and which promotes increased immune responses to future challenges with the same or different stimuli (24, 25). Meanwhile in ASCVD, neutrophils contribute to different stages and clinical manifestation of atherosclerosis (26) and literature also suggests that inflammation-adapted hematopoietic stem and progenitor cells (HSPCs) may contribute to the disease pathogenesis (27-29). As such, recent consensus between European Federation of Periodontology and World Heart Federation includes neutrophil hyper-responsiveness as one of mechanisms to explain epidemiological association between periodontitis and ASCVD (2).

Mechanisms linking periodontitis to ASCVD and the effect of periodontitis treatment in improving the surrogate markers of ASCVD in attempt to show causal interactions between the two diseases have been extensively explored (2, 14). However, the causal mechanistic pathways between these two common non-communicable diseases are yet to be fully understood. In this review, we aim to critically address the roles of neutrophils in linking periodontitis to ASCVD. Systemic inflammation triggered by different causes including periodontitis may drive inflammatory adaptation of HSPCs and trained granulopoiesis in the bone marrow resulting in increased production of neutrophils with a hyper-responsive phenotype (12, 30) This systemic inflammation-driven modification of granulopoiesis can contribute to atherosclerosis in a stage-dependent manner. However, although the periodontitis treatment successfully achieves the resolution in periodontal tissue site and improves

surrogate markers of ASCVD, studies reveal that the hyper-responsive function in neutrophils may persist (23, 31). Therefore, novel approaches to target neutrophils by manipulating their numbers and functions are warranted in periodontitis treatment and to mitigate its impact on ASCVD.

2 The link between periodontitis and ASCVD

The impact of the treatment of periodontitis on cardiovascular outcomes and surrogate markers of ASCVD has been extensively explored. Patients with periodontitis exhibited an increased risk of coronary and cerebrovascular events compared to periodontally healthy individuals. These findings may not apply to the whole population as influenced by the demographic characteristics, individuals, studies, and case definition of periodontitis (2, 32). In the ARIC study on 6736 dentate participants with 299 incidents of ischemic stroke revealed that seven periodontal profile classes that were used to assess the participants were associated with increased risk of cardioembolic and thrombotic stroke subtypes compared to periodontally healthy participants. The assessment in this study was based on seven tooth-clinical parameters resulting in seven different periodontal profile classes and the greater class indicates more severe form of periodontitis (33). Lastly, based on the 1999–2010 Taiwanese National Health Insurance Research Database involving 393,745 patients with periodontitis and 393,745 non-periodontitis individuals demonstrated a significantly increased incidence of arterial fibrillation in patients with periodontitis compared to controls (34). The findings from all studies had been adjusted for wide potential confounders indicating that periodontitis is an independent risk for an increased risk of ASCVD events.

Treatment of periodontitis may influence the progression of ASCVD. The study involving 511,630 periodontitis patients and 208,713 individuals without periodontitis from The Longitudinal Database of Taiwan's National Health Insurance demonstrated that patients receiving dental prophylaxis had lower hazard ratio of acute myocardial infarction compared to periodontally healthy controls suggesting an almost 10% reduction in the risk of a new ASCVD event (35). These improvements were not observed across all types of periodontitis and controls suggesting a difference in host susceptibility and response even after the treatment of peridontitis when looking at future incidence of ASCVD events (36).

Consistent evidence suggests that periodontitis is associated with higher blood pressure and endothelial dysfunction (2, 37-39). A recent review and subsequent meta-analysis of intervention studies confirmed that the management of periodontitis can be a novel non-drug approach for the treatment of hypertension. The evidence is still inconclusive as whether the treatment of periodontitis influences blood pressure even in absence of hypertension (40). The basis of this improvement in vascular function could be linked to the effect of the treatment of periodontitis in improving endothelial function as assessed by FMD (41-44). A possible mechanism by which local periodontal treatment improves endothelial function in periodontitis patients is endothelial nitric oxide synthase/nitric oxide (eNOS/NO) activation. NO is mainly produced by eNOS in endothelial cells and induces the relaxation of smooth muscle cells (45, 46). Meanwhile, IL-6, TNFα, IL-1β and CRP directly reduced eNOS at both mRNA and protein levels in human endothelial cells (47-49). As a result, NO bioavailability in the serum is reduced leading to endothelial dysfunction and periodontitis is associated with this surrogate marker of ASCVD (50, 51). Hepatocytes are the major producer of CRP triggered by IL-6 and IL-1β stimulation, while TNFα also upregulated CRP production in human coronary artery smooth muscle cells (52-55). A recent meta-analysis reveals a progressive CRP level reduction up to 6 months in patients with periodontitis following effective treatment (37). This was also associated with reduction of reduced serum IL-6, TNFα, and IL-1β in patients with periodontitis compared to baseline (56-59). Collectively, these findings suggest that reduced levels of

CRP, IL-6, TNFα, and IL-1β after treatment of periodontitis could restore eNOS activity and NO bioavailability resulting in improved endothelial dysfunction.

3 Periodontitis as a trigger of systemic inflammation

Periodontitis and ASCVD share similar hallmark of inflammatory mechanisms (60), genetic (2, 61), and common risk factors (62). However, a significant body of evidence supports an independent association between periodontitis and ASCVD following adjustment for confounders and shared risk factors (63, 64). This independent association can be explained by the capability of periodontitis to trigger a low-grade, but consistent systemic inflammation which may contribute to the development of ASCVD (65). Patients with periodontitis exhibit an elevated level of systemic pro-inflammatory mediators which include CRP, TNFα, IL-1β, IL-6, as well as increased neutrophil numbers in the blood (2, 14, 66-69). A retrospective study involving 60,174 participants revealed that even after the adjustment of confounders, participants with periodontitis were 1.59 times more likely to have ASCVD (63). Previously, an 8-year prospective cohort study involving 11,869 participants also showed that those reporting poor-oral hygiene had an enhanced risk of CVD event as well as an elevated level of CRP and fibrinogen in the serum (70).

Systemic inflammation triggered by periodontitis potentially occurs as a result of bacterial dissemination or periodontal tissue-derived inflammatory mediator leakage to blood circulation. The ulceration of the epithelium owing to local periodontium inflammation along with the support of its rich vascularization may provide greater access for bacteria and their endotoxins such as lipopolysaccharides (LPS) to circulation, leading to bacteremia (13). This event was reported in patients with periodontitis during mastication, toothbrushing, and dental scaling (10, 11). The bacteremia would induce inflammatory alteration in the endothelium which are enhanced expression of adhesion molecules and production of pro-inflammatory cytokines. With regards to the spillover of inflammatory mediators to the bloodstream, this can affect vascular tissues as well as other distant organs including liver which then may initiate acute-phase response (14).

4 Inflammation and neutrophils: implications for atherosclerosis

Atherosclerosis is a cause for myocardial infarction, ischemic cardiomyopathy, and ischemic stroke that contribute to the majority of death in the population worldwide (4). Arterial wall damage due to blood lipid profile imbalance, oscillating shear stress, and pro-inflammatory mediators initiate this underlying ASCVD pathology. The subsequent process is endothelial cell activation in arterial tissue, myeloid cell adhesion to endothelium, and infiltration into the arterial intima (26). At the late stage of atherosclerosis, inflammatory cell accumulation, lipoprotein deposition, and cellular debris buildup are responsible for the arterial plaque formation followed by the plaque instability leading to atherosclerotic plaque rupture. Atherosclerosis is a decades-long process with many stages of evolution and evidence suggests that neutrophils involve in different stages of atherosclerosis (**Figure 1)** (26).

The onset of atherosclerosis is characterized by endothelial dysfunction which induces neutrophil recruitment to the endothelium. Specifically, the dysfunction up-regulates the expression of various endothelial cell adhesion molecules including E-selectin, P-selectin, and intracellular adhesion molecule-1 (ICAM-1) (71). Platelets then deliver CCL5, the primary ligand of CCR5 on the endothelium and promote cathepsin G secretion by neutrophils resulting in the firm adhesion to and accumulation of the cells in the endothelium (72, 73). Further, neutrophils aggravate the endothelial dysfunction by secreting reactive oxygen species (ROS), azurocidin, proteinase 3, cathelicidin, and

cathepsin G in the arterial lumen (26, 74). ROS and proteases activate and dysregulate endothelial cell layer and degrade the underlying extracellular matrix, enabling leukocyte infiltration and low-density lipoprotein (LDL) extravasation (26). Azurocidin also contributes to increasing endothelial permeability (26, 74-76), while azurocidin, proteinase 3, cathelicidin, α-defensin and, cathepsin G all promote myeloid cell recruitment facilitating monocyte entry to the atherosclerotic lesion (74, 77-82).

The progression of the lesion continues following the aggravation of endothelial dysfunction by neutrophils where macrophage activation and foam cell formation occur in the arterial intima. Neutrophil-derived granule proteins, cathelicidin, and α-defensin activate macrophages toward pro-inflammatory state (M1 macrophage phenotype). Neutrophils also secrete myeloperoxidase (MPO) to generate oxygen radicals that oxidize apolipoprotein B, a protein structure in LDL (82). Subsequently, macrophages take up this oxidized LDL (oxLDL) resulting in foam cell formation (83). Moreover, NETs stimulate macrophages by turning on transcription factors encoding IL-6 and IL-1β. These cytokines promote the differentiation of Th17 cells, which in turn amplify neutrophils recruitment in the lesion (84). At this stage, as a result of sustained myeloid cell recruitment and foam cell generation, the atheroma becomes pronounced.

In the late stage of atherosclerosis, neutrophils destabilize the atherosclerotic plaque. Mechanistically, activated vascular smooth muscle cells (VSMCs) in advanced atherosclerotic lesion induce neutrophil chemotaxis and secrete CCL7 that stimulates NET release. One of NET cytotoxic components, histone H4, disrupts the integrity of VSMC plasma membrane leading to the cell lysis (85). Moreover, endotoxemia in mice model of atherosclerosis revealed that leukotriene B4-induced neutrophil recruitment to atherosclerotic plaques induces collagen degradation and VSMC lysis leading to the feature of plaque instability (86). In eroded human plaques, neutrophils colocalized with toll-like receptor 2 of endothelial cells and in vitro experiment showed that co-culture of neutrophil with endothelial cells potentiates endothelial stress and apoptosis, resulting in endothelial cell detachment followed by luminal endothelial cell desquamation (plaque erosion) (19). Lastly, NETs also involve during endothelial erosion as the disruption of NETs by either peptidyl arginine deiminase 4 (PAD4) gene knockout or DNase I treatment in atherosclerotic mice attenuated endothelial disintegration and endothelial cell apoptosis (87).

Neutrophils play dual roles which are both adverse and favorable for cardiac tissue repair following myocardial infarction (**Figure 2A**). Tissue necrosis/ischemia post-acute myocardial infarction releases alarmins and inflammatory signals that attract neutrophils to the site of infarction (88). Activated neutrophils secrete ROS, proteases, NETs, and IL-1β. Granulopoiesis stimulated by IL-1β leads to neutrophil accumulation in the injured site which is detrimental to the remodeling of ischemic area resulting in eventual heart failure (89, 90). Following neutrophil accumulation, monocytes and monocyte-derived macrophages infiltrate the infarcted site to phagocytose cell debris and apoptotic neutrophils, leading to the activation of cardiac repair (88). Intriguingly, neutrophils also contribute to this cardiac healing as their damage-associated molecular patterns (DAMPs), such as neutrophil gelatinase-associated lipocalin (NGAL) and S100A8/A9, stimulate macrophages to shift towards reparative phenotypes (91, 92). These anti-inflammatory macrophages aid the resolution of inflammation (88). Neutrophils also secrete annexin A1 that stimulates pro-angiogenic macrophage polarization. These macrophage phenotypes release vascular endothelial growth factor A (VEGFA) to support angiogenesis in the ischemic site of the myocardium (93). Beside cardiac tissue repair, neutrophils aggravate other atherosclerosis complications, namely ischemic stroke (**Figure 2B**). Following episode of ischemic stroke, dying neurons attract neutrophils to the area to release ROS and elastase that enhance endothelial cell dysfunction and permeability. NETs promote

thrombus growth, thereby increasing stroke volume. Finally, neutrophils increase neuronal cell death in a process that is likely to involve NETs (26, 94).

5 Impact of periodontitis-induced systemic inflammation on bone marrow activity and subsequent circulating neutrophil alterations: Plausible mechanisms

5.1 Modulation of HSPCs in the bone marrow: a key role for periodontitis-induced systemic inflammation

Trained immunity can be initiated in the bone marrow via sustained epigenetic, metabolic, and transcriptional adaptations in HSPCs, leading to enhanced myeloid-biased differentiation and production of increased numbers of trained myeloid cells including neutrophils (trained myelopoiesis/granulopoiesis) (95, 96). This training is based on the ability of HSPCs to sense numerous inflammatory cues in response to hematopoietic stress such as systemic inflammation (12, 96). HSPCs can directly sense pathogen-associated molecular patterns (PAMPs), such as LPS, via their pattern recognition receptors (PRRs) such as toll-like receptors (for example, TLR-4 for sensing LPS). Regarding the direct mechanism, in the context of periodontitis-induced bacteremia, it could be envisioned that systemically disseminated periodontal pathogens or their products (for example LPS or lipopeptides) may also reach the bone marrow resulting in innate immune training on HSPCs. On the other hand, indirect activation relies on specialized cells residing in either the peripheral tissue or bone marrow affecting the hematopoietic system through the release of cytokines (97, 98).

In addition to direct sensing of pathogens, cytokines and growth factors derived from both the bone marrow niche and peripheral tissue can mediate indirect adaptation of HSPCs (97, 98). IL-1β promotes myeloid differentiation and self-renewal of HSPCs as chronic administration of this cytokine elevates the number of myeloid-bias HSPCs. This is also consistent with the recent finding that enhanced myelopoiesis of HSPCs in a mouse model of β-glucan or experimental periodontitis-induced trained immunity is mediated by IL-1β (95, 99). TNFα exhibits different actions on HSPCs as it promotes both the survival and myeloid differentiation of HSCs while inducing the apoptosis of myeloid progenitors (100). Increased level of IL-6 in the bone marrow niche promotes myelopoiesis indicated by an elevated number of multipotent progenitors (MPPs) and common myeloid progenitors (CMPs) (101). Type 1 IFN mediates trained granulopoiesis in mice following β-glucan treatment resulting in the production of neutrophils with an enhanced ROS-dependent anti-tumor phenotype (102). IFNγ promotes HSC self-renewal and myeloid differentiation in mouse model of repeated *Mycobacterium avium* infection (103). Granulocyte colony stimulating factor (G-CSF) is a key growth factor that drives granulopoiesis. Specifically, G-CSF produced by monocytes in the bone marrow niche is responsible for HSPC mobilization from the bone marrow to circulation (104). Moreover, in emergency granulopoiesis, G-CSF promotes the expansion and granulocyte lineage specification of granulocyte-monocyte progenitors (GMPs) (105).

Systemic inflammation indicated by elevated levels of TNFα, IL-1β, IL-6, and IFNγ in the serum is clinically present in patient with periodontitis (66, 67). Plasma IFNα, a type 1 IFN is also higher in periodontitis patients compared to healthy controls (106). However, the role of type 1 IFN-mediated trained granulopoiesis in inducing hyper-responsive neutrophils in periodontitis patients needs to be addressed experimentally. Recent evidence indicates an increased level of serum G-CSF in ligature-induced periodontitis mice (107). Fibroblasts in the periodontal tissue contribute to the release of G-CSF during periodontal inflammation resulting in the promotion of granulopoiesis (108). Based on the cumulative evidence of the effect of cytokines and growth factors on HSPCs and clinical studies mentioned above, it could be supported that periodontitis-associated systemic inflammation may

modulate HSPCs toward trained granulopoiesis (**Table 1**). This notion was recently confirmed in a preclinical model. Specifically, it was shown that ligature-induced periodontitis (LIP)-associated systemic inflammation leads to maladaptive innate immune training in the bone marrow (i.e., generating inflammatory memory) and the generation of increased numbers of hyper-responsive neutrophils; these populate oral and non-oral tissues and promote the emergence of inflammatory comorbidities, as exemplified by the periodontitis-arthritis axis (99). This is consistent with available clinical observations as outlined below. Intriguingly, the transplantation of bone marrow from LIP-subjected mice to healthy recipient mice resulted in increased severity of arthritis in the latter, as compared to transplantation of bone marrow from periodontally healthy mice (99). The implication of this finding (if a similar phenomenon is confirmed in humans) is that clinicians should take inflammatory memory in the bone marrow into consideration when selecting appropriate donors for hematopoietic transplantation (99).

Indeed, the notion that periodontitis might trigger adaptation of HSPCs is supported by clinical imaging studies using ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT). One study revealed that periodontal inflammation was associated with hematopoietic activity in the bone marrow, and arterial inflammation. Further, the authors used mediation path analysis to show that the relationship between periodontal and arterial inflammation was significantly mediated by bone marrow activity (109). More recently, another study using the same cohort of 304 participants as the aforementioned study, showed that periodontal inflammation (as determined by ^{18}F-FDG-PET/CT) is independently correlated with not only increased arterial inflammation but also increased risk of future cardiovascular events (64). In addition, another study harnessing the same imaging technique also reported a trend for increased periodontal inflammation and femur bone marrow activity in patients with periodontitis (relative to controls), albeit no differences were observed in vascular inflammation between the two groups (110). The lack of apparent differences in this study is likely attributed (a) to the small sample size (14 participants as opposed to >300 participants in the above-discussed studies) and (b) to the fact that participants with severe periodontal disease were under supportive periodontal therapy, which could have mitigated inflammation and associated parameters, including surrogate markers of ASCVD (discussed below) (110). Moreover, the control group also included individuals with mild periodontitis, which could also impact systemic inflammation and hematopoietic tissue activity, thus reducing potential differences as compared to the experimental group.

5.2 Alteration of circulating neutrophils in periodontitis and its putative effect on atherosclerosis

Patients with periodontitis exhibit an elevated numbers of neutrophils and altered phenotypes presenting hyper-reactive features in cellular functions (discussed below) (21-23, 68, 69, 110-117). These include increased level of ROS production in response to fMLP, PMA, or periodontal pathogens (21-23, 102, 111-115), elevated TNFα production following the stimulation with the periodontal pathogen, *Fusobacterium nucleatum*, in vitro (116), and elevated neutrophil elastase levels linked to periodontal tissue destruction (117, 118). The systemic effect of this alteration has been recently confirmed in an experimental study with mice and experimental periodontitis that exhibited hyper-inflammatory neutrophil response following the exposure of secondary peritonitis compared to mice without periodontitis (119).

These altered features in neutrophils present a hallmark of trained myelopoiesis which includes increased numbers of myeloid cells with enhanced inflammatory responsiveness (24, 95). As such,

these changes may implicate the response of future inflammatory stimuli that drive certain pathological process such as atherosclerosis.

Neutrophils that are increased in number and hyper-responsiveness due to periodontitis can contribute to any stage of this ASCVD pathology. Further research should address this hypothesis experimentally. However, it is still interesting to speculate that periodontitis-induced neutrophil alteration contributes to the association between periodontitis to ASCVD because of several clinical studies that back this notion. An elevated number of neutrophils in peripheral blood may increase the risk of ASCVD in periodontitis patients because neutrophil counts from peripheral blood is positively correlated with ASCVD risk (120, 121). Moreover, periodontitis patients consistently present endothelial dysfunction which is a key feature in ASCVD (2). This disturbed vascular function and an elevated neutrophil ROS production triggered by periodontitis can aggravate the initiation of atherosclerosis. The latter may also potentiate the progression of atherosclerosis, particularly in oxidizing LDL. Hyper-responsiveness of neutrophils characterized by excessive production of neutrophil elastase and ROS in periodontitis patients could also contribute to the late stage of atherosclerosis as both hyper-reactive features induce endothelial apoptosis resulting in endothelial desquamation (plaque erosion), fibrous cap thinning and plaque ruptures. Finally, whereas the production of NETs by circulating neutrophils have been shown to be comparable between patients with periodontitis and healthy controls, plasma NET degradation was lower in patient with periodontitis than controls suggesting an impaired NET degradation process in the plasma in periodontitis patients (122). This impairment might favor atherosclerosis complication, especially in post-ischemic stroke where NET accumulation is known to promote thrombus formation and expands stroke volume. In this context, a case-control study revealed that periodontitis was an independent predictor of poor outcome in post-ischemic stroke patients (123).

6 Impact of periodontitis treatment on neutrophils

Whereas the treatment of periodontitis improves the surrogate markers of ASCVD, its long-term cardiovascular-protective effect is uncertain. A reduction of several serum inflammatory markers that is expected to reduce the risk of ASCVD is not observed in serum IFNγ and IL-10 because both markers remain unchanged following periodontitis treatment (40). As these markers are predominantly generated and released to blood circulation by inflammatory cells, it is interesting to speculate that the successful treatment indeed achieves the resolution of periodontal inflammation, but circulating inflammatory cells still retain their periodontitis-induced altered phenotypes. The latter is supported by clinical studies that investigated the functions of peripheral blood-derived neutrophils and monocytes in periodontitis patients after the treatment. The hyper-responsiveness of these myeloid cells persisted as the levels of cytokines produced by the cells in response to pathogen or LPS stimulation were comparable between neutrophils from patients before and after periodontal treatment (31, 116). Similarly, a longitudinal study on the circulating neutrophil profiles of patients with periodontitis showed that neutrophils (as a proportion of total cells isolated from peripheral blood of periodontitis patients) did not change between baseline (prior to treatment) and after 3-, 6-, and 12-months post-periodontitis treatment (124). The retained neutrophil phenotypes might be as a result of trained myelopoiesis induced by periodontitis-triggered systemic inflammation. In murine experiments, long-lasting changes in myelopoiesis were observed following either microbial- or sterile-induced inflammation (29, 95). Similarly, recent report shows that upon LIP resolution, HSPCs in the bone marrow retain a myeloid differentiation bias. (99).

7 Targeting neutrophils as a novel therapeutic approach: Dual benefit on periodontitis and ASCVD

The targeted therapeutic approach for ASCVD has been established focusing on the reduction of inflammation (**Table 2**). The first clinical trial, CANTOS, in attempt to close the gap between pre-clinical studies and clinical practice, was conducted providing evidence that reducing inflammation could be relevant to treat atherosclerosis in human. In this trial, the anti-IL-1β antibody, Canakinumab were administered subcutaneously to individuals with a sustained acute myocardial infarction. The trial reveals a significant reduction in the rate of recurrent cardiovascular events, hospitalization for heart failure, and heart failure-associated death (125, 126). However, the adverse events in the group receiving Canakinumab was a significant more death due to infection or sepsis compared to placebo group (125). Moreover, oral administration of Colchicine in patients with a recent myocardial infarction significantly reduced the risk of ischemic cardiovascular events (127). The benefits of Colchicine were also observed in patients with chronic coronary disease (128). Unfortunately, patients in Colchicine group showed higher incident of pneumonia and non-cardiovascular caused death than those in placebo group (127, 128). Other clinical studies on targeting inflammation in ASVD treatment are reviewed elsewhere (129). These clinical trials indicate that therapies with alternative strategies are needed to achieve outcomes in which the benefits outweigh the risks.

Beside the necessity of different strategies to reduce the risk-benefit ratio, a refined understanding on the potential role of periodontitis-induced neutrophil alteration in the pathology of ASCVD and its retained phenotypes following successful periodontitis treatment highlight the importance of selectively targeting neutrophils during the treatment of periodontitis. This therapeutic approach is warranted to mitigate potential impacts of periodontitis on ASCVD. The main endpoint of this intervention is to either manipulate the number of neutrophils and/or their functional activities. We only discuss about the approaches to block neutrophil recruitment and prevent NET-driven inflammation, while other neutrophil-targeted therapeutics was extensively reviewed elsewhere (130).

While the results of neutrophil recruitment blockage are promising in preclinical studies, clinical trials exhibit unsuccessful outcomes due to the redundancy of signals during neutrophil recruitment and off-target effects caused by receptor cross-linking. Recent studies provide strategies to overcome such difficulties. The combined inhibition of several endothelial cell molecules interrupts redundant signals that recruit neutrophils (131). Intravenous injection of nanoparticle carrying small interfering RNAs (siRNAs) that target endothelial adhesion molecules including ICAM-1, E-, P-selectin, and vascular cellular adhesion molecule-1 (VCAM-1) decreased leukocyte recruitment to ischemic myocardium in mice model of post-myocardial infarction (131). Moreover, specific blockage of neutrophils that traffic to certain vascular tissue requires refined understanding on recruitment patters in particular sites (16). A neutrophil granule protein, cathepsin G promotes myeloid cell adhesion to only arterial, but not microvascular tissue (73). Indeed, antibody-assisted cathepsin G neutralization in atherosclerotic mice model specifically alleviated neutrophil recruitment to carotid artery resulting in reduced atherosclerotic plaque sizes. The similar treatment, however, did not affect neutrophil adhesion in lung microcirculation following LPS-induced lung inflammation model in mice (73). Further, the elucidation of heteromeric interactions between neutrophil-derived human neutrophil peptide-1 (HNP1) and platelet-borne CCL5 that are required to stimulate monocyte adhesion via CCR5 ligation allows to design a peptide that can disturb these neutrophil-platelet interactions. This specifically designed short peptide alleviated inflammation in mouse model of myocardial infarction (20). Finally, inducing endogenous inhibitors in leukocytes can overcome integrin activation by

chemokine, thereby suppressing myeloid cell adhesion. For example, growth differentiation factor-15 (GDF-15) and annexin A1 inhibited chemokine-induced β2 integrin activation then subsequently reduced neutrophil recruitment in mouse model of chronic inflammation (72, 132). Moreover, recombinant developmental endothelial locus-1 (DEL-1), the first identified endogenous inhibitor of the leukocyte adhesion cascade (133), inhibited neutrophil recruitment in mouse and non-human primate models (134, 135).

Important roles of NETs in both atherosclerosis progression (atherogenesis, plaque destabilization and erosion) and complication (atherothrombosis) stand out as a potential therapeutic target to manipulate cardiovascular inflammation. Peptidyl arginine deiminase 4 (PAD4) citrullinates histone to disrupt electrostatic bond in nucleosomes decondensing chromatin that leads NET release (136). Cl-amidine, a PAD inhibitor administered intravenously in atherosclerotic mice model prevented NET formation leading to reduced atherosclerotic lesion area and thrombosis (137). However, the mechanism of NETosis in mouse is different with human as ex vivo experiments of human neutrophils showed that PMA-induced NETosis of the cells was not affected following PAD4 inhibitor, Cl-amidine (138). Meanwhile, DNase 1 treatment might be considered to mitigate ASCVD complication, thrombosis due to NET deposition in vascular lumen. This notion is supported by a study where the treatment protected mice from deep vein thrombosis following inferior vena cava stenosis model (139). A reduction in lesion size was also observed in atherosclerosis mice model after DNase injections (84). In human, DNAse 1 treatment to eliminate NET deposition might need a combination with other substance as DNAse 1 alone was not adequate to degrade NETs in vitro (140). Lastly, NET chromatin can stimulate macrophage by activating AIM2 inflammation causing the release of IL-8 and IL-1β in atherosclerotic lesion. The use of AIM2 inhibitor could also attenuate ASCVD complication because reduced plaque vulnerability through the thickening of fibrous cap was observed in atherosclerotic mice model after the treatment of AIM2 inhibitor (141). ApoE-deficient mice on 6-week high fat diet and injected with anti-chromatin antibodies also showed a reduced plaque area per lumen suggesting the potential of chromatin blockage to hinder atherosclerosis (142). The use of substances to block NET in human should be implemented with caution because studies about pro-inflammatory features of NETs in human is still conflicting as in vitro NET clearance by human macrophages did not induce pro-inflammatory cytokines (140), but NET transfection to mouse macrophages did (143).

Targeting neutrophils as an approach to mitigate the potential impacts periodontitis on ASCVD is appealing considering numerous efforts outlined previously. This targeted strategy can have direct effects on ASCVD, but also reduce periodontitis which triggers inflammation that primes neutrophils for ASCVD pathology. However, safety and specificity issues are challenges that need to be solved as neutrophils interact with other myeloid lineages (130). The solution for the former concern is that the intervention should consider the therapeutic window, where the attenuation of inflammatory processed mediated by neutrophils does not interfere with neutrophil capacity during host defence (130). Recent study reveals that the use of antibodies to inhibit NET-derived histones reduced the amplification of NET-induced inflammation rather completely blocked NETosis or inflammation (142). A small number of studies presented strategies to obtain the specificity to therapeutically target neutrophil. For example, the conjugation of siRNA targeting Bruton's tyrosine kinase (BTK) to F(ab')2 fragment of an anti-neutrophil monoclonal antibody specifically targeted alveolar neutrophils in acute lung injury mice model. This treatment was administered locally using a technique called intranasal instillation (144). Meanwhile, others harness nanoparticle technology for the enhancement of specificity. One of them reported that neutrophils adhered to activated endothelium engulfed albumin nanoparticles carrying piceatannol leading to inactivation of these adherent neutrophils that consequently prevented vascular inflammation (145). In addition, another

report exhibited lipid-based nanoparticles that were successfully incorporated with identified peptides that interact with neutrophil-specific surface marker, CD177 (146).

The clinical trial in targeting neutrophils to lower ASCVD risk is still lacking. However, two randomized clinical trials reported that metoprolol provides a cardio-protective effect following acute myocardial infarction which is one of atherosclerosis complications (**Table 2**). Specifically, intravenous administration of metoprolol reduced infarct size and improved cardiac function in patient with acute myocardial infarction (147, 148). Studies using myocardial infarct mice model reveal the mechanism of this protection in which metoprolol attenuates neutrophil migration and infiltration by impairing the neutrophil-platelet interaction that is crucial during early phases of neutrophil recruitment (149, 150). Another drug candidate, AZD5069, a CXCR2 antagonist could be a potent drug candidate in treating patients with advanced atherosclerotic lesion. CXCR2 is a chemokine receptor 2 in neutrophils that regulates neutrophil migration and the inhibition of this receptor successfully prevented neutrophil recruitment to the site of inflammation (151). Adverse effect on neutrophil function was not observed and no safety concerns were raised in participants receiving oral administration of AZD5069 (152). The CICADA trial was proposed to investigate the effect of CXCR2 antagonist (administered orally) on coronary flow, structure, and function in patients with coronary heart disease (153).

Mechanical intervention in periodontitis management with adjunctive neutrophil-targeted therapeutic approach could also provide benefit to reduce periodontal-induced systemic inflammation that can implicate all stages of atherosclerosis. A phase IIa clinical trial of complement C3 inhibitor, AMY-101, summarized in **Table 2** shows promising results in reducing local inflammation in periodontal tissue (154). The pharmacological blockade of the central complement component, C3, inhibits downstream activation of the anaphylatoxin C3a and C5a receptors (C3aR and C5aR, respectively), which have been shown to induce inflammatory bone loss in a preclinical model (155). C5aR and TLR2 coactivation in neutrophils contributes to oral microbiota dysbiosis leading to overt periodontal inflammation and subsequent periodontal tissue destruction (156). Local injection of AMY-101 to the gingiva reduced gingival inflammation without adverse events warranting phase III clinical trials as further investigations (154, 157). Importantly, AMY-101 significantly reduced the gingival crevicular levels of MMP-8 and MMP-9 (153), which are the major neutrophil-derived proteases and considered as biomarkers of periodontal tissue destruction (158). Meanwhile, other local neutrophil-targeted treatments including resolvin E1, developmental endothelial locus-1, and milk fat globule epidermal growth factor 8 are still in pre-clinical studies. These proteins reduce neutrophil recruitment to the site of inflammation and prevent animal models of ligature-induced periodontitis (135, 159, 160). In addition, resolving E1 also promotes neutrophil apoptosis and its clearance (efferocytosis) resulting in the resolution of periodontal inflammation (158). Focusing on the termination of periodontitis may prevent its systemic impact which is heightened systemic inflammation. These strategies of local intervention potentially further improve the current periodontal treatment in providing a sustained protection to cardiovascular health.

8 Conclusion

Periodontitis triggers systemic inflammation which could modulate hematopoietic tissue activity in the bone marrow resulting in trained myelopoiesis. Clinical studies demonstrating an increased numbers as well as enhanced inflammatory responsiveness in neutrophils back this notion. The evidence of persistent elevated neutrophil numbers and their altered phenotypes, despite the resolution of periodontitis following local treatment, also supports the speculation that periodontitis-induced systemic inflammation can induce long-term myelopoiesis bias, a hallmark of innate

immune training in the bone marrow. The quantitative and qualitative alterations in neutrophils may contribute to all stages of the ASCVD pathology, atherosclerosis (**Figure 3**). Although clinical intervention studies suggest that periodontal therapy improves surrogate marker of ASCVD, long term effect of this oral treatment to maintain such improvement and convincing evidence that successful periodontitis treatment can reduce the risk or incidence of ASCVD are yet to be investigated. Meanwhile, targeting neutrophils are warranted to improve local periodontal therapy eliminating periodontitis effectively that can reduce periodontitis-triggered systemic inflammation and to reverse the periodontitis-induced neutrophil changes. Such targeted approaches can be harnessed as a direct treatment for ASCVD and indirect intervention of the disease through the reduction of heightened systemic inflammation triggered by periodontitis.

9 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

10 Author Contributions

RAI wrote the original draft. RAI, STC, GH, VP, JED, and FD provided critical revisions to the article. All authors contributed to the article and approved the submitted version.

11 Acknowledgments

This work was undertaken at UCL Biomedical Research Centre which receives funding from the UK National Institute for Health and Care Research. GH receives a grant from the US National Institutes of Health (DE031206). RAI receives funding from the Indonesia Endowment Fund for Education (S-1692/LPDP.4/2019 – LPDP – Indonesia Scholarship). The figures were created using Biorender.com

12 Reference

1. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y, Ali F. Atherosclerotic Cardiovascular Disease: a Review of Initiators and Protective Factors. *Inflammopharmacology* (2016) 24(1):1-10. doi: 10.1007/s10787-015-0255-y
2. Sanz M, Marco del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and Cardiovascular Diseases: Consensus Report. *J Clin Periodontol* (2020) 47(3):268-88. doi: 10.5334/gh.400
3. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update from the GBD 2019 Study. *J Am Coll Cardiol* (2020) 76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010
4. Libby P. The Biology of Atherosclerosis Comes Full Circle: Lessons for Conquering Cardiovascular Disease. *Nat Rev Cardiol* (2021) 18(10):683-4. doi: 10.1038/s41569-021-00609-1
5. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal Diseases. *Nat Rev Dis Primers* (2017) 3(1):17038. doi: 10.1038/nrdp.2017.38
6. Graziani F, Music L, Bozic D, Tsakos G. Is Periodontitis and Its treatment Capable of Changing the Quality of Life of a Patient? *Br Dent J* (2019) 227(7):621-5. doi: 10.1038/s41415-019-0735-3
7. Kassebaum N, Bernabé E, Dahiya M, Bhandari B, Murray C, Marcenes W. Global Burden of Severe Periodontitis in 1990-2010: a Systematic Review and Meta-Regression. *J Dent Res* (2014) 93(11):1045-53. doi: 10.1177/0022034514552491

8. Dye BA. Global Periodontal Disease Epidemiology. *Periodontol 2000* (2012) 58(1):10-25. doi: 10.1111/j.1600-0757.2011.00413.x
9. Baehni P, Tonetti M, Group 1 of the European Workshop on Periodontology. Conclusions and Consensus Statements on Periodontal Health, Policy and Education in Europe: a Call for Action–Consensus View 1: Consensus report of the 1st European Workshop on Periodontal Education. *Eur J Dent Educ* (2010) 14:2-3. doi: 10.1111/j.1600-0579.2010.00619.x
10. Geerts SO, Nys M, De Mol P, Charpentier J, Albert A, Legrand V, et al. Systemic Release of Endotoxins Induced by Gentle Mastication: Association with Periodontitis Severity. *J Periodontol* (2002) 73(1):73-8. doi: 10.1902/jop.2002.73.1.73
11. Forner L, Larsen T, Kilian M, Holmstrup P. Incidence of Bacteremia After Chewing, Tooth Brushing and Scaling in Individuals with Periodontal Inflammation. *J Clin Periodontol* (2006) 33(6):401-7. doi: 10.1111/j.1600-051X.2006.00924.x
12. Hajishengallis G, Chavakis T. Local and Systemic Mechanisms Linking Periodontal Disease and Inflammatory Comorbidities. *Nat Rev Immunol* (2021) 21(7):426-440. doi: 0.1038/s41577-020-00488-6
13. Konkel JE, O'Boyle C, Krishnan S. Distal Consequences of Oral Inflammation. *Front Immunol* (2019) 10:1403. doi: 10.3389/fimmu.2019.01403
14. Schenkein HA, Papapanou PN, Genco R, Sanz M. Mechanisms Underlying the Association Between Periodontitis and Atherosclerotic Disease. *Periodontol 2000* (2020) 83(1):90-106. doi: 10.1111/prd.12304
15. Rosales C. Neutrophil: a Cell with Many Roles in Inflammation or Several Cell Types? *Front Physiol* (2018) 9:113. doi: 10.3389/fphys.2018.00113
16. Soehnlein O, Steffens S, Hidalgo A, Weber C. Neutrophils as Protagonists and Targets in Chronic Inflammation. *Nat Rev Immunol* (2017) 17(4):248-61. doi: 10.1038/nri.2017.10
17. Eskan MA, Jotwani R, Abe T, Chmelar J, Lim J-H, Liang S, et al. The Leukocyte Integrin Antagonist Del-1 Inhibits IL-17-mediated Inflammatory Bone Loss. *Nat Immunol* (2012) 13(5):465-73. doi: 10.1038/ni.2260
18. Dutzan N, Kajikawa T, Abusleme L, Greenwell-Wild T, Zuazo CE, Ikeuchi T, et al. A Dysbiotic Microbiome Triggers T(H)17 Cells to Mediate Oral Mucosal Immunopathology in Mice and Humans. *Sci Transl Med* (2018) 10(463):eaat0797. doi: 10.1126/scitranslmed.aat0797
19. Quillard T, Araújo HA, Franck G, Shvartz E, Sukhova G, Libby P. TLR2 and Neutrophils Potentiate Endothelial Stress, Apoptosis and Detachment: Implications for Superficial Erosion. *Eur Heart J* (2015) 36(22):1394-404. doi: 10.1093/eurheartj/ehv044
20. Alard J-E, Ortega-Gomez A, Wichapong K, Bongiovanni D, Horckmans M, Megens RT, et al. Recruitment of Classical Monocytes Can be Inhibited by Disturbing Heteromers of Neutrophil HNP1 and Platelet CCL5. *Sci Transl Med* (2015) 7(317):317ra196-317ra196. doi: 10.1126/scitranslmed.aad5330
21. Matthews J, Wright H, Roberts A, Cooper P, Chapple I. Hyperactivity and Reactivity of Peripheral Blood Neutrophils in Chronic Periodontitis. *Clin Exp Immunol* (2007) 147(2):255-64. doi: 10.1111/j.1365-2249.2006.03276.x
22. Matthews J, Wright H, Roberts A, Ling-Mountford N, Cooper P, Chapple I. Neutrophil Hyper-responsiveness in Periodontitis. *J Dent Res* (2007) 86(8):718-22. doi: 10.1177/154405910708600806
23. Ling MR, Chapple IL, Matthews JB. Neutrophil Superoxide Release and Plasma C-reactive Protein Levels Pre-and Post-periodontal therapy. *J Clin Periodontol* (2016) 43(8):652-8. doi: 10.1111/jcpe.12575

24. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining Trained Immunity and Its role in Health and Disease. *Nat Rev Immunol* (2020) 20(6):375-88. doi: 10.1038/s41577-020-0285-6
25. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonça LE, Pacis A, et al. BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. *Cell* (2018) 172(1-2):176-90.e19. doi: 10.1016/j.cell.2017.12.031
26. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as Regulators of Cardiovascular Inflammation. *Nat Rev Cardiol* (2020) 17(6):327-40. doi: 10.1038/s41569-019-0326-7
27. Chavakis T, Wielockx B, Hajishengallis G. Inflammatory Modulation of Hematopoiesis: Linking Trained Immunity and Clonal Hematopoiesis with Chronic Disorders. *Ann Rev Physiol* (2021) 84:183-207. doi: 10.1146/annurev-physiol-052521-013627
28. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden C, Li Y, et al. Metabolic Induction of Trained Immunity through the Mevalonate Pathway. *Cell* (2018) 172(1-2):135-46.e9. doi: 10.1016/j.cell.2017.11.025
29. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, et al. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* (2018) 172(1-2):162-75.e14. doi: 10.1016/j.cell.2017.12.013
30. Poller WC, Nahrendorf M, Swirski FK. Hematopoiesis and Cardiovascular Disease. *Circ Res* (2020) 126(8):1061-85. doi: 10.1161/CIRCRESAHA.120.315895
31. Radvar M, Tavakkol-Afshari J, Bajestan MN, Naseh MR, Arab HR. The Effect of Periodontal Treatment on IL-6 Production of Peripheral Blood Monocytes in Aggressive Periodontitis and Chronic Periodontitis Patients. *Iran J Immunol* (2008) 5(2):100-6.
32. Dietrich T, Sharma P, Walter C, Weston P, Beck J. The Epidemiological Evidence Behind the Association Between Periodontitis and Incident Atherosclerotic Cardiovascular Disease. *J Periodontol* (2013) 84(4 Suppl):S70-84. doi: 10.1902/jop.2013.134008
33. Sen S, Giamberardino LD, Moss K, Morelli T, Rosamond WD, Gottesman RF, et al. Periodontal Disease, Regular Dental Care Use, and Incident Ischemic Stroke. *Stroke* (2018) 49(2):355-362. doi: 10.1161/STROKEAHA.117.018990
34. Chen D-Y, Lin C-H, Chen Y-M, Chen H-H. Risk of Atrial Fibrillation or Flutter Associated with Periodontitis: a Nationwide, Population-based, Cohort Study. *PLoS One* (2016) 11(10):e0165601. doi: 10.1371/journal.pone.0165601
35. Lee Y-L, Hu H-Y, Chou P, Chu D. Dental Prophylaxis Decreases the Risk of Acute Myocardial Infarction: a Nationwide Population-based Study in Taiwan. *Clin Interv Aging* (2015) 10:175-82. doi: 10.2147/CIA.S67854
36. Holmlund A, Lampa E, Lind L. Poor Response to Periodontal Treatment May Predict Future Cardiovascular Disease. *J Dent Res* (2017) 96(7):768-773. doi: 10.1177/0022034517701901
37. Machado V, Botelho J, Escalda C, Hussain SB, Luthra S, Mascarenhas P, et al. Serum C-reactive Protein and Periodontitis: A Systematic Review and Meta-analysis. *Front Immunol* (2021) 12:706432. doi: 10.3389/fimmu.2021.706432
38. Muñoz Aguilera E, Suvan J, Buti J, Czesnikiewicz-Guzik M, Barbosa Ribeiro A, Orlandi M, et al. Periodontitis is Associated with Hypertension: a Systematic Review and Meta-Analysis. *Cardiovasc Res* (2020) 116(1):28-39. doi: 10.1093/cvr/cvz201.
39. Muñoz Aguilera E, Suvan J, Orlandi M, Miró Catalina Q, Nart J, D'Aiuto F. Association Between Periodontitis and Blood Pressure Highlighted in Systemically Healthy Individuals: Results from a Nested Case-Control Study. *Hypertension* (2021) 77(5):1765-1774. doi: 10.1161/HYPERTENSIONAHA.120.16790
40. Sharma S, Sridhar S, Mcintosh A, Messow C-M, Aguilera EM, Del Pinto R, et al. Periodontal Therapy and Treatment of Hypertension–Alternative to the Pharmacological Approach. A

Systematic Review and Meta-analysis. *Pharmacol Res* (2021) 166:105511. doi: 10.1016/j.phrs.2021.105511

41. Orlandi M, Suvan J, Petrie A, Donos N, Masi S, Hingorani A, et al. Association Between Periodontal Disease and Its Treatment, Flow-Mediated Dilatation and Carotid Intima-Media Thickness: a Systematic Review and Meta-Analysis. *Atherosclerosis* (2014) 236(1):39-46. doi: 10.1016/j.atherosclerosis.2014.06.002
42. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al. Treatment of Periodontitis and Endothelial Function. *N Engl J Med* (2007) 356(9):911-20. doi: 10.1056/NEJMoa063186
43. D'Aiuto F, Gkranias N, Bhowruth D, Khan T, Orlandi M, Suvan J, et al. Systemic Effects of Periodontitis Treatment in Patients with Type 2 Diabetes: a 12 Month, Single-centre, Investigator-Masked, Randomised Trial. *Lancet Diabetes Endocrinol* (2018) 6(12):954-965. doi: 10.1016/S2213-8587(18)30038-X
44. Czesnikiewicz-Guzik M, Osmenda G, Siedlinski M, Nosalski R, Pelka P, Nowakowski D, et al. Causal Association between Periodontitis and Hypertension:Evidence from Mendelian Randomization and a Randomized Controlled Trial of Non-Surgical Periodontal Therapy. *Eur Heart J* (2019) 40(42):3459-3470. doi: 10.1093/eurheartj/ehz646
45. Stuehr DJ. Mammalian Nitric Oxide Synthases. *Biochim Biophys Acta* (1999) 1411(2-3):217-30. doi: 10.1016/s0005-2728(99)00016-x
46. Palmer RM, Ferrige AG, Moncada S. Nitric Oxide Release Accounts for the Biological Activity of Endothelium-derived Relaxing Factor. *Nature* (1987) 327(6122):524-6. doi: 10.1038/327524a0
47. Yoshizumi M, Perrella MA, Burnett JC, Jr., Lee ME. Tumor Necrosis Factor Downregulates an Endothelial Nitric Oxide Synthase mRNA by Shortening Its Half-Life. *Circ Res* (1993) 73(1):205-9. doi: 10.1161/01.res.73.1.205
48. Bhagat K, Vallance P. Inflammatory Cytokines Impair Endothelium-Dependent Dilatation in Human Veins In Vivo. *Circulation* (1997) 96(9):3042-7. doi: 10.1161/01.cir.96.9.3042
49. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive Protein Decreases eNOS Expression and Bioactivity in Human Aortic Endothelial Cells. *Circulation* (2002) 106(12):1439-41. doi: 10.1161/01.cir.0000033116.22237.f9
50. Clapp BR, Hingorani AD, Kharbanda RK, Mohamed-Ali V, Stephens JW, Vallance P, et al. Inflammation-induced Endothelial Dysfunction Involves Reduced Nitric Oxide Bioavailability and Increased Oxidant Stress. *Cardiovasc Res* (2004) 64(1):172-8. doi: 10.1016/j.cardiores.2004.06.020
51. Higashi Y, Goto C, Jitsuiki D, Umemura T, Nishioka K, Hidaka T, et al. Periodontal Infection is Associated with Endothelial Dysfunction in Healthy Subjects and Hypertensive Patients. *Hypertension* (2008) 51(2):446-53. doi: 10.1161/HYPERTENSIONAHA.107.101535
52. Baumann M, Baumann H, Fey G. Molecular Cloning, Characterization and Functional Expression of the Rat Liver Interleukin 6 Receptor. J Biol Chem (1990) 265(32):19853-62.
53. Nesbitt JE, Fuller GM. Differential Regulation of Interleukin-6 Receptor and Gp130 Gene Expression in Rat Hepatocytes. *Mol Biol Cell* (1992) 3(1):103-12. doi: 10.1091/mbc.3.1.103
54. Calabró P, Willerson JT, Yeh ET. Inflammatory Cytokines Stimulated C-Reactive Protein Production by Human Coronary Artery Smooth Muscle Cells. *Circulation* (2003) 108(16):1930-2. doi: 10.1161/01.CIR.0000096055.62724.C5
55. Zhang D, Jiang SL, Rzewnicki D, Samols D, Kushner I. The Effect of Interleukin-1 on C-Reactive Protein Expression in Hep3B Cells is Exerted at the Transcriptional Level. *Biochem J* (1995) 310 (Pt 1)(Pt 1):143-8. doi: 10.1042/bj3100143
56. Zhou QB, Xia WH, Ren J, Yu BB, Tong XZ, Chen YB, et al. Effect of Intensive Periodontal Therapy on Blood Pressure and Endothelial Microparticles in Patients With Prehypertension

and Periodontitis: A Randomized Controlled Trial. *J Periodontol* (2017) 88(8):711-22. doi: 10.1902/jop.2017.160447

57. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The Effect of Periodontal Treatment on Serum Leptin, Interleukin-6, and C-Reactive Protein. J Periodontol (2010) 81(8):1118-23. doi: 10.1902/jop.2010.090741
58. Türer Ç C, Durmuş D, Balli U, Güven B. Effect of Non-Surgical Periodontal Treatment on Gingival Crevicular Fluid and Serum Endocan, Vascular Endothelial Growth Factor-A, and Tumor Necrosis Factor-Alpha Levels. *J Periodontol* (2017) 88(5):493-501. doi: 10.1902/jop.2016.160279
59. Vijayakumar S, Koshi E, Sadasivan A, Indhuja RS, Vallabhan CG. Effect of Nonsurgical Periodontal Therapy on Serum Levels of Interleukin-1β and Interleukin-8 in Smokers and Nonsmokers with Chronic Periodontitis. *J Pharm Bioallied Sci* (2020) 12(Suppl 1):S313-s8. doi: 10.4103/jpbs.JPBS_93_20
60. Paul O, Arora P, Mayer M, Chatterjee S. Inflammation in Periodontal Disease: Possible Link to Vascular Disease. *Front Physiol* (2021) 14;11:609614. doi: 10.3389/fphys.2020.609614.
61. Loos BG, Van Dyke TE. The Role of Inflammation and Genetics in Periodontal Disease. *Periodontol 2000* (2020) 83(1):26-39. doi: 10.1111/prd.12297
62. Herrera D, Molina A, Buhlin K, Klinge B. Periodontal diseases and association with atherosclerotic disease. Periodontol 2000 (2020) 83(1):66-89. doi: 10.1111/prd.12302
63. Beukers NG, van der Heijden GJ, van Wijk AJ, Loos BG. Periodontitis is an Independent Risk Indicator for Atherosclerotic Cardiovascular Diseases Among 60 174 Participants in a Large Dental School in the Netherlands. *J Epidemiol Community Health* (2017) 71(1):37-42. doi: 10.1136/jech-2015-206745
64. Van Dyke TE, Kholy KE, Ishai A, Takx RA, Mezue K, Abohashem SM, et al. Inflammation of the Periodontium Associates with Risk of Future Cardiovascular Events. J Periodontol (2021) 92(3):348-58. doi: 10.1002/JPER.19-0441
65. Moutsopoulos NM, Madianos PN. Low-grade Inflammation in Chronic Infectious Diseases: Paradigm of Periodontal Infections. *Ann N Y Acad Sci* (2006) 1088:251-64. doi: 10.1196/annals.1366.032
66. Górska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madaliński K. Relationship between Clinical Parameters and Cytokine Profiles in Inflamed Gingival Tissue and Serum Samples from Patients with Chronic Periodontitis. *J Clin Periodontol* (2003) 30(12):1046-52. doi: 10.1046/j.0303-6979.2003.00425.x
67. Loos BG, Craandijk J, Hoek FJ, Dillen PMWv, Van Der Velden U. Elevation of Systemic Markers Related to Cardiovascular Diseases in the Peripheral Blood of Periodontitis Patients. *J Periodontol* (2000) 71(10):1528-34. doi: 10.1902/jop.2000.71.10.1528
68. Botelho J, Machado V, Hussain SB, Zehra SA, Proença L, Orlandi M, et al. Periodontitis and Circulating Blood Cell Profiles: a Systematic Review and Meta-Analysis. *Exp Hematol* (2021) 93:1-13. doi: 10.1016/j.exphem.2020.10.001
69. Irwandi RA, Kuswandani SO, Harden S, Marletta D, D'Aiuto F. Circulating inflammatory cell profiling and periodontitis: A systematic review and meta-analysis. *J Leukoc Biol* (2022) 111(5):1069-1096. doi: 10.1002/JLB.5RU1021-524R
70. de Oliveira C, Watt R, Hamer M. Toothbrushing, Inflammation, and Risk of Cardiovascular Disease: Results from Scottish Health Survey. *BMJ* (2010) 27;340:c2451. doi: 10.1136/bmj.c2451
71. Zarbock A, Ley K. Mechanisms and Consequences of Neutrophil Interaction with the Endothelium. *Am J Pathol* (2008) 172(1):1-7. doi: 10.2353/ajpath.2008.070502

72. Drechsler M, de Jong R, Rossaint J, Viola JR, Leoni G, Wang JM, et al. Annexin A1 Counteracts Chemokine-induced Arterial Myeloid Cell Recruitment. *Circ Res* (2015) 116(5):827-35. doi: 10.1161/CIRCRESAHA.116.305825
73. Ortega-Gomez A, Salvermoser M, Rossaint J, Pick R, Brauner J, Lemnitzer P, et al. Cathepsin G Controls Arterial but Not Venular Myeloid Cell Recruitment. *Circulation* (2016) 134(16):1176-88. doi: 10.1161/CIRCULATIONAHA.116.024790
74. Soehnlein O. Multiple Roles for Neutrophils in Atherosclerosis. *Circ Res* (2012) 110(6):875-88. doi: 10.1161/CIRCRESAHA.111.257535
75. Di Gennaro A, Kenne E, Wan M, Soehnlein O, Lindbom L, Haeggström JZ. Leukotriene B4-induced Changes in Vascular Permeability Are Mediated by Neutrophil Release of Heparin-binding Protein (HBP/CAP37/azurocidin). *FASEB J* (2009) 23(6):1750-7. doi: 10.1096/fj.08-121277
76. Rasmuson J, Kenne E, Wahlgren M, Soehnlein O, Lindbom L. Heparinoid Sevuparin Inhibits Streptococcus-induced Vascular Leak Through Neutralizing Neutrophil-derived Proteins. *FASEB J* (2019) 33(9):10443-52. doi: 10.1096/fj.201900627R
77. Chertov O, Ueda H, Xu LL, Tani K, Murphy WJ, Wang JM, et al. Identification of Human Neutrophil-derived Cathepsin G and Azurocidin/CAP37 as Chemoattractants for Mononuclear Cells and Neutrophils. *J Exp Med* (1997) 186(5):739-47. doi: 10.1084/jem.186.5.739
78. Taekema-Roelvink MEJ, Kooten CV, Kooij SV, Heemskerk E, Daha MR. Proteinase 3 Enhances Endothelial Monocyte Chemoattractant Protein-1 Production and Induces Increased Adhesion of Neutrophils to Endothelial Cells by Upregulating Intercellular Cell Adhesion Molecule-1. *J Am Soc Nephrol* (2001) 12(5):932-40. doi: 10.1681/ASN.V125932
79. De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, et al. LL-37, the Neutrophil Granule- and Epithelial Cell-derived Cathelicidin, Utilizes Formyl Peptide Receptor-like 1 (FPRL1) as A Receptor to Chemoattract Human Peripheral Blood Neutrophils, Monocytes, and T Cells. *J Exp Med* (2000) 192(7):1069-74. doi: 10.1084/jem.192.7.1069
80. Chaly YV, Paleolog EM, Kolesnikova TS, Tikhonov, II, Petratchenko EV, Voitenok NN. Neutrophil Alpha-Defensin Human Neutrophil Peptide Modulates Cytokine Production in Human Monocytes and Adhesion Molecule Expression in Endothelial Cells. *Eur Cytokine Netw* (2000) 11(2):257-66.
81. Sun R, Iribarren P, Zhang N, Zhou Y, Gong W, Cho EH, et al. Identification of Neutrophil Granule Protein Cathepsin G as A Novel Chemotactic Agonist for the G protein-coupled Formyl Peptide Receptor. *J Immunol* 2004 173(1):428-36. doi: 10.4049/jimmunol.173.1.428
82. Carr AC, McCall MR, Frei B. Oxidation of LDL by Myeloperoxidase and Reactive Nitrogen Species: Reaction Pathways and Antioxidant Protection. *Arterioscler Thromb Vasc Biol* (2000) 20(7):1716-23. doi: 10.1161/01.atv.20.7.1716
83. Bekkering S, Quintin J, Joosten LA, van der Meer JW, Netea MG, Riksen NP. Oxidized Low-Density Lipoprotein Induces Long-term Proinflammatory Cytokine Production and Foam Cell Formation via Epigenetic Reprogramming of Monocytes. *Arterioscler Thromb Vasc Biol* (2014) 34(8):1731-8. doi: 10.1161/ATVBAHA.114.303887
84. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Neutrophil Extracellular Traps License Macrophages for Cytokine Production in Atherosclerosis. *Science* (2015) 17;349(6245):316-20. doi: 10.1126/science.aaa8064
85. Silvestre-Roig C, Braster Q, Wichapong K, Lee EY, Teulon JM, Berrebeh N, et al. Externalized Histone H4 Orchestrates Chronic Inflammation by Inducing Lytic Cell Death. *Nature* (2019) 569(7755):236-40. doi: 10.1038/s41586-019-1167-6
86. Mawhin MA, Tilly P, Zirka G, Charles AL, Slimani F, Vonesch JL, et al. Neutrophils Recruited by Leukotriene B4 Induce Features of Plaque Destabilization during Endotoxaemia. *Cardiovasc Res* (2018) 114(12):1656-66. doi: 10.1093/cvr/cvy130

87. Franck G, Mawson TL, Folco EJ, Molinaro R, Ruvkun V, Engelbertsen D, et al. Roles of PAD4 and NETosis in Experimental Atherosclerosis and Arterial Injury: Implications for Superficial Erosion. *Circ Res* (2018) 123(1):33-42. doi: 10.1161/CIRCRESAHA.117.312494
88. Gopalkrishna S, Johnson J, Jaggers RM, Dahdah A, Murphy AJ, Hanssen NM, et al. Neutrophils in Cardiovascular Disease: Warmongers, Peacemakers or Both?. *Cardiovasc Res* (2021) cvab302. doi: 10.1093/cvr/cvab302
89. Nagareddy PR, Sreejit G, Abo-Aly M, Jaggers RM, Chelvarajan L, Johnson J, et al. NETosis is required for S100A8/A9-induced Granulopoiesis After Myocardial Infarction. *Arterioscler Thromb Vasc Biol* (2020) 40(11):2805-07. doi: 10.1161/ATVBAHA.120.314807
90. Sreejit G, Abdel-Latif A, Athmanathan B, Annabathula R, Dhyani A, Noothi SK, et al. Neutrophil-derived S100A8/A9 Amplify Granulopoiesis After Myocardial Infarction. *Circulation* (2020) 31;141(13):1080-94. doi: 10.1161/CIRCULATIONAHA.119.043833
91. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, et al. Neutrophils Orchestrate Post-myocardial Infarction Healing by Polarizing Macrophages Towards a Reparative Phenotype. *Eur Heart J* (2017) 14;38(3):187-97. doi: 10.1093/eurheartj/ehw002
92. Marinković G, Koenis DS, de Camp L, Jablonowski R, Graber N, de Waard V, et al. S100A9 Links Inflammation and Repair in Myocardial Infarction. *Circ Res* (2020) 14;127(5):664-76. doi: 10.1161/CIRCRESAHA.120.315865
93. Ferraro B, Leoni G, Hinkel R, Ormanns S, Paulin N, Ortega-Gomez A, et al. Pro-angiogenic Macrophage Phenotype to Promote Myocardial Repair. *J Am Coll Cardiol* (2019) 18;73(23):2990-3002. doi: 10.1016/j.jacc.2019.03.503
94. Allen C, Thornton P, Denes A, McColl BW, Pierozynski A, Monestier M, et al. Neutrophil Cerebrovascular Transmigration Triggers Rapid Neurotoxicity Through Release of Proteases Associated with Decondensed DNA. *J Immunol* (2012) 189(1):381-92. doi: 10.4049/jimmunol.1200409
95. Mitroulis I, Ruppova K, Wang B, Chen LS, Grzybek M, Grinenko T, et al. Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell* (2018) 172(1-2):147-61.e12. doi: 10.1016/j.cell.2017.11.034
96. Chavakis T, Mitroulis I, Hajishengallis G. Hematopoietic Progenitor Cells as Integrative Hubs for Adaptation to and Fine-tuning of Inflammation. *Nat Immunol* (2019) 20(7):802-11. doi: 10.1038/s41590-019-0402-5
97. King KY, Goodell MA. Inflammatory Modulation of HSCs: Viewing the HSC as a Foundation for the Immune Response. *Nat Rev Immunol* (2011) 11(10):685-92. doi: 10.1038/nri3062
98. Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol* (2014) 14(5):302-14. doi: 10.1038/nri3660
99. Li X, Wang H, Yu X, Saha G, Kalafati L, Ioannidis C, Mitroulis I, Netea MG, Chavakis T, Hajishengallis G. Maladaptive Innate Immune Training of Myelopoiesis Links Inflammatory Comorbidities. *Cell* (2022)185(10):1709-27.e18. doi: 10.1016/j.cell.2022.03.043
100. Yamashita M, Passegué E. TNF-α Coordinates Hematopoietic Stem Cell Survival and Myeloid Regeneration. *Cell Stem Cell* (2019) 25(3):357-372.e7. doi: 10.1016/j.stem.2019.05.019
101. Schürch CM, Riether C, Ochsenbein AF. Cytotoxic CD8+ T cells Stimulate Hematopoietic Progenitors by Promoting Cytokine Release from Bone Marrow Mesenchymal Stromal Cells. *Cell Stem Cell* (2014) 3;14(4):460-72. doi: 10.1016/j.stem.2014.01.002
102. Kalafati L, Kourtzelis I, Schulte-Schrepping J, Li X, Hatzioannou A, Grinenko T, et al. Innate Immune Training of Granulopoiesis Promotes Anti-Tumor Activity. *Cell* (2020) 183(3):771-85.e12. doi: 10.1016/j.cell.2020.09.05
103. Matatall KA, Jeong M, Chen S, Sun D, Chen F, Mo Q, et al. Chronic Infection Depletes Hematopoietic Stem Cells Through Stress-induced Terminal Differentiation. *Cell Rep* (2016) 17(10):2584-95. doi: 10.1016/j.celrep.2016.11.031

104. Christopher MJ, Rao M, Liu F, Woloszynek JR, Link DC. Expression of the G-CSF Receptor in Monocytic Cells is Sufficient to Mediate Hematopoietic Progenitor Mobilization by G-CSF in Mice. *J Exp Med* (2011) 208(2):251-60. doi: 10.1084/jem.20101700
105. Hérault A, Binnewies M, Leong S, Calero-Nieto FJ, Zhang SY, Kang Y-A, et al. Myeloid Progenitor Cluster Formation Drives Emergency and Leukaemic Myelopoiesis. *Nature* (2017) 544(7648):53-8. doi: 10.1038/nature21693
106. Wright HJ, Matthews JB, Chapple IL, Ling-Mountford N, Cooper PR. Periodontitis Associates with a Type 1 IFN Signature in Peripheral Blood Neutrophils. *J Immunol* (2008) 181(8):5775-84. doi: 10.4049/jimmunol.181.8.5775
107. Yu H, Zhang T, Lu H, Ma Q, Zhao D, Sun J, et al. Granulocyte Colony-Stimulating Factor (G-CSF) Mediates Bone Resorption in Periodontitis. *BMC Oral Health*. (2021) 21(1):299. doi: 10.1186/s12903-021-01658-1
108. Hajishengallis G, Chavakis T, Hajishengallis E, Lambris JD. Neutrophil Homeostasis and Inflammation: Novel Paradigms from Studying Periodontitis. *J Leukoc Biol* (2015) 98(4):539-48. doi: 10.1189/jlb.3VMR1014-468R
109. Ishai A, Osborne MT, El Kholy K, Takx RA, Ali A, Yuan N, et al. Periodontal Disease Associates with Arterial Inflammation via Potentiation of a Hematopoietic-Arterial Axis. *JACC Cardiovasc Imaging* (2019) 12(11 Pt 1):2271-73. doi: 10.1016/j.jcmg.2019.05.015
110. Noz MP, Plachokova AS, Smeets EM, Aarntzen EH, Bekkering S, Vart P, et al. An Explorative Study on Monocyte Reprogramming in the Context of Periodontitis In Vitro and In Vivo. *Front Immunol* (2021) 12:695227. doi: 10.3389/fimmu.2021.69522
111. Fredriksson MI. Effect of Priming in Subpopulations of Peripheral Neutrophils from Patients with Chronic Periodontitis. *J Periodontol* (2012) 83(9):1192-9. doi: 10.1902/jop.2012.110584
112. Johnstone AM, Koh A, Goldberg MB, Glogauer M. A Hyperactive Neutrophil Phenotype in Patients with Refractory Periodontitis. *J Periodontol* (2007) 78(9):1788-94. doi: 10.1902/jop.2007.070107
113. Kim KY, Kim M-K, Choi YS, Kim YC, Jo A-r, Rhyu I-C, et al. A Hyperactive Neutrophil Phenotype in Aggresive Periodontitis. *Int J Oral Biol* (2013) 37(2):69-75.
114. Dias IH, Chapple IL, Milward M, Grant MM, Hill E, Brown J, et al. Sulforaphane Restores Cellular Glutathione Levels and Reduces Chronic Periodontitis Neutrophil Hyperactivity in Vitro. *PLoS One* (2013) 8(6):e66407. doi: 10.1371/journal.pone.0066407
115. Žilinskas J, Žekonis J, Žekonis G, Šadzevičienė R, Sapragonienė M, Navickaitė J, et al. Inhibition of Peripheral Blood Neutrophil Oxidative Burst in Periodontitis Patients with a Homeopathic Medication Traumeel S. *Med Sci Monit* (2011) 17(5):Cr284-91. doi: 10.12659/msm.881769
116. Ling MR, Chapple IL, Matthews JB. Peripheral Blood Neutrophil Cytokine Hyper-Reactivity in Chronic Periodontitis. *Innate Immun* (2015) 21(7):714-25. doi: 10.1177/1753425915589387
117. Figueredo C, Gustafsson A, Åsman B, Bergström K. Expression of Intracellular Elastase Activity in Peripheral Neutrophils from Patients with Adult Periodontitis. *J Clin Periodontol* (2000) 27(8):572-7. doi: 10.1034/j.1600-051x.2000.027008572.x
118. Guentsch A, Puklo M, Preshaw PM, Glockmann E, Pfister W, Potempa J, et al. Neutrophils in Chronic and Aggressive Periodontitis in Interaction with Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. *J Periodontal Res* (2009) 44(3):368-77. doi: 10.1111/j.1600-0765.2008.01113.x
119. Fine N, Chadwick JW, Sun C, Parbhakar KK, Khoury N, Barbour A, Goldberg M, Tenenbaum HC, Glogauer M. Periodontal Inflammation Primes the Systemic Innate Immune Response. *J Dent Res* (2021) 100(3):318-325. doi: 10.1177/0022034520963710.
120. Welsh C, Welsh P, Mark PB, Celis-Morales CA, Lewsey J, Gray SR, et al. Association of Total and Differential Leukocyte Counts with Cardiovascular Disease and Mortality in the UK

Biobank. *Arterioscler Thromb Vasc Biol* (2018) 38(6):1415-1423. doi: 10.1161/ATVBAHA.118.310945

121. Lassale C, Curtis A, Abete I, van der Schouw YT, Verschuren WM, Lu Y. Elements of the Complete Blood Count Associated with Cardiovascular Disease Incidence: Findings from the EPIC-NL Cohort Study. *Sci Rep* (2018) 8(1):3290. doi: 10.1038/s41598-018-21661-x
122. White P, Sakellari D, Roberts H, Risafi I, Ling M, Cooper P, et al. Peripheral Blood Neutrophil Extracellular Trap Production and Degradation in Chronic Periodontitis. *J Clin Periodontol* (2016) 43(12):1041-1049. doi: 10.1111/jcpe.12628
123. Leira Y, Rodríguez-Yáñez M, Arias S, López-Dequidt I, Campos F, Sobrino T, et al. Periodontitis as a Risk Indicator and Predictor of Poor Outcome for Lacunar Infarct. *J Clin Periodontol* (2019) 46(1):20-30. doi: 10.1111/jcpe.13032
124. Medara N, Lenzo JC, Walsh KA, Reynolds EC, O'Brien-Simpson NM, Darby IB. Peripheral Neutrophil Phenotypes During Management of Periodontitis. *J Periodontal Res* (2021) 56(1):58-68. doi: 10.1111/jre.12793
125. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* (2017) 377(12):1119-1131. doi: 10.1056/NEJMoa1707914
126. Everett BM, Cornel JH, Lainscak M, Anker SD, Abbate A, Thuren T, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention of Hospitalization for Heart Failure. *Circulation* (2019) 139(10):1289-99. doi: 10.1161/CIRCULATIONAHA.118.038010
127. Tardif J-C, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *N Engl J Med* (2019) 381(26):2497-505. doi: 10.1056/NEJMoa1912388
128. Nidorf SM, Fiolet ATL, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, et al. Colchicine in Patients with Chronic Coronary Disease. *N Engl J Med* (2020) 383(19):1838-47. doi: 10.1056/NEJMoa2021372
129. Soehnlein O, Libby P. Targeting Inflammation in Atherosclerosis — From Experimental Insights to the Clinic. *Nat Rev Drug Discov* (2021) 20(8):589-610. doi: 10.1038/s41573-021-00198-1
130. Németh T, Sperandio M, Mócsai A. Neutrophils as Emerging Therapeutic Targets. *Nat Rev Drug Discov* (2020) 19(4):253-75. doi: 10.1038/s41573-019-0054-z
131. Sager HB, Dutta P, Dahlman JE, Hulsmans M, Courties G, Sun Y, et al. RNAi Targeting Multiple Cell Adhesion Molecules Reduces Immune Cell Recruitment and Vascular Inflammation After Myocardial Infarction. *Sci Transl Med* (2016) 8(342):342ra80. doi: 10.1126/scitranslmed.aaf1435
132. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, et al. GDF-15 is an Inhibitor of Leukocyte Integrin Activation Required for Survival After Myocardial Infarction in Mice. *Nat Med* (2011) 17(5):581-8. doi: 10.1038/nm.2354
133. Choi EY, Orlova VV, Fagerholm SC, Nurmi SM, Zhang L, Ballantyne CM, et al. Regulation of LFA-1-Dependent Inflammatory Cell Recruitment by Cbl-b and 14-3-3 Proteins. *Blood* (2008) 111(7):3607-14. doi: 10.1182/blood-2007-07-103077
134. Eskan MA, Jotwani R, Abe T, Chmelar J, Lim JH, Liang S, et al. The Leukocyte Integrin Antagonist Del-1 Inhibits IL-17-Mediated Inflammatory Bone Loss. *Nat Immunol* (2012) 13(5):465-73. doi: 10.1038/ni.2260
135. Shin J, Maekawa T, Abe T, Hajishengallis E, Hosur K, Pyaram K, et al. DEL-1 restrains osteoclastogenesis and inhibits inflammatory bone loss in nonhuman primates. *Sci Transl Med.* 2015;7(307):307ra155.
136. Papayannopoulos V. Neutrophil Extracellular Traps in Immunity and Disease. *Nat Rev Immunol* (2018) 18(2):134-147. doi: 10.1038/nri.2017.105

137. Knight JS, Luo W, O'Dell AA, Yalavarthi S, Zhao W, Subramanian V, et al. Peptidylarginine Deiminase Inhibition Reduces Vascular Damage and Modulates Innate Immune Responses in Murine Models of Atherosclerosis. *Circ Res* (2014) 114(6):947-56. doi: 10.1161/CIRCRESAHA.114.303312
138. Kenny EF, Herzig A, Krüger R, Muth A, Mondal S, Thompson PR, et al. Diverse Stimuli Engage Different Neutrophil Extracellular Trap Pathways. *Elife* (2017) 6:e24437. doi: 10.7554/eLife.24437
139. Brill A, Fuchs T, Savchenko A, Thomas G, Martinod K, De Meyer S, et al. Neutrophil Extracellular Traps Promote Deep Vein Thrombosis in Mice. *J Thromb Haemost* (2012) 10(1): 136–144. doi: 10.1111/j.1538-7836.2011.04544.x
140. Farrera C, Fadeel B. Macrophage Clearance of Neutrophil Extracellular Traps is a Silent Process. *J Immunol* (2013) 191(5):2647-56 doi: 10.4049/jimmunol.1300436
141. Paulin N, Viola JR, Maas SL, de Jong R, Fernandes-Alnemri T, Weber C, et al. Double-Strand DNA Sensing Aim2 Inflammasome Regulates Atherosclerotic Plaque Vulnerability. *Circulation* (2018) 138(3):321-3. doi: 10.1161/CIRCULATIONAHA.117.033098.
142. Tsourouktsoglou TD, Warnatsch A, Ioannou M, Hoving D, Wang Q, Papayannopoulos V. Histones, DNA, and Citrullination Promote Neutrophil Extracellular Trap Inflammation by Regulating the Localization and Activation of TLR4. *Cell Rep* (2020) 31(5):107602. doi: 10.1016/j.celrep.2020.107602
143. Apel F, Andreeva L, Knackstedt LS, Streeck R, Frese CK, Goosmann C, et al. The Cytosolic DNA Sensor cGAS Recognizes Neutrophil Extracellular Traps. *Sci Signal* (2021) 14(673). doi: doi: 10.1126/scisignal.aax7942
144. Krupa A, Fol M, Rahman M, Stokes KY, Florence JM, Leskov IL, et al. Silencing Bruton's Tyrosine Kinase in Alveolar Neutrophils Protects Mice from LPS/Immune Complex-Induced Acute Lung Injury. *Am J Physiol Lung Cell Mol Physiol* (2014) 307(6):L435-48. doi: 10.1152/ajplung.00234.2013
145. Wang Z, Li J, Cho J, Malik AB. Prevention of Vascular Inflammation by Nanoparticle Targeting of Adherent Neutrophils. *Nature Nanotechnol* (2014) 9(3):204-10. doi: 10.1038/nnano.2014.17
146. Miettinen HM, Gripentrog JM, Lord CI, Nagy JO. CD177-Mediated Nanoparticle Targeting of Human and Mouse Neutrophils. *PloS One* (2018) 13(7):e0200444. doi: 10.1371/journal.pone.0200444
147. Ibanez B, Macaya C, Sánchez-Brunete V, Pizarro G, Fernández-Friera L, Mateos A, et al. Effect of Early Metoprolol on Infarct Size in ST-Segment-Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention: the Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) trial. *Circulation* (2013) 128(14):1495-503. doi: 10.1161/CIRCULATIONAHA.113.003653
148. Pizarro G, Fernández-Friera L, Fuster V, Fernández-Jiménez R, García-Ruiz JM, García-Álvarez A, et al. Long-term Benefit of Early Pre-Reperfusion Metoprolol Administration in Patients With Acute Myocardial Infarction: Results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction). *J Am Coll Cardiol* (2014) 63(22):2356-62. doi: 10.1016/j.jacc.2014.03.014
149. García-Prieto J, Villena-Gutiérrez R, Gómez M, Bernardo E, Pun-García A, García-Lunar I, et al. Neutrophil Stunning by Metoprolol Reduces Infarct Size. *Nat Commun* (2017) 8:14780. doi: 10.1038/ncomms14780
150. Clemente-Moragón A, Gómez M, Villena-Gutiérrez R, Lalama DV, García-Prieto J, Martínez F, et al. Metoprolol Exerts a Non-Class Effect Against Ischaemia-Reperfusion Injury by Abrogating Exacerbated Inflammation. *Eur Heart J* (2020) 41(46):4425-40. doi: 10.1093/eurheartj/ehaa733

151. Herz J, Sabellek P, Lane TE, Gunzer M, Hermann DM, Doeppner TR. Role of Neutrophils in Exacerbation of Brain Injury After Focal Cerebral Ischemia in Hyperlipidemic Mice. *Stroke* (2015) 46(10):2916-25. doi: 10.1161/STROKEAHA.115.010620
152. Jurcevic S, Humfrey C, Uddin M, Warrington S, Larsson B, Keen C. The Effect of a Selective CXCR2 Antagonist (AZD5069) on Human Blood Neutrophil Count and Innate Immune Functions. *Br J Clin Pharmacol* (2015) 80(6):1324-36. doi: 10.1111/bcp.12724
153. Joseph JP, Reyes E, Guzman J, O'Doherty J, McConkey H, Arri S, et al. CXCR2 Inhibition - a novel approach to treating CoronAry heart DiseAse (CICADA): study protocol for a randomised controlled trial. *Trials* (2017) 18(1):473. doi: 10.1186/s13063-017-2210-2
154. Hasturk H, Hajishengallis G, Lambris JD, Mastellos DC, Yancopoulou D. Phase IIa Clinical Trial of Complement C3 Inhibitor AMY-101 in Adults with Periodontal Inflammation. *J Clin Invest* (2021) 131(23):e152973. doi: 10.1172/JCI152973
155. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, et al. Low-Abundance Biofilm Species Orchestrates Inflammatory Periodontal Disease Through the Commensal Microbiota and Complement. *Cell Host Microbe* (2011) 10(5):497-506. doi: 10.1016/j.chom.2011.10.006
156. Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K, et al. Porphyromonas Gingivalis Manipulates Complement and TLR Signaling to Uncouple Bacterial Clearance from Inflammation and Promote Dysbiosis. *Cell Host Microbe* (2014) 15(6):768-78. doi: 10.1016/j.chom.2014.05.012
157. Hajishengallis G, Hasturk H, Lambris JD. C3-targeted Therapy in periodontal Disease: Moving Closer to the Clinic. *Trends Immunol* (2021) 42(10):856-64. doi: 10.1016/j.it.2021.08.001
158. Räisänen IT, Lähteenmäki H, Gupta S, Grigoriadis A, Sahni V, Suojanen J, et al. An aMMP-8 Point-of-Care and Questionnaire Based Real-Time Diagnostic Toolkit for Medical Practitioners. *Diagnostics (Basel)* (2021) 11(4):711. doi: 10.3390/diagnostics11040711
159. Lee CT, Teles R, Kantarci A, Chen T, McCafferty J, Starr JR, et al. Resolvin E1 Reverses Experimental Periodontitis and Dysbiosis. *J Immunol* (2016) 197(7):2796-806. doi: 10.4049/jimmunol.1600859
160. Kajikawa T, Meshikhes F, Maekawa T, Hajishengallis E, Hosur KB, Abe T, et al. Milk Fat Globule Epidermal Growth Factor 8 Inhibits Periodontitis in Non-Human Primates and Its Gingival Crevicular Fluid Levels Can Differentiate Periodontal Health from Disease in Humans. *J Clin Periodontol* (2017) 44(5):472-83. doi: 10.1111/jcpe.12707

Figure captions

Figure 1. Stage-dependent role of neutrophils in atherosclerosis.

Figure 2. Neurophils in ASCVD complications.

Figure 3. Periodontitis-induced systemic inflammation, inflammatory modulation of bone marrow progenitor cells, and implications for atherosclerosis.

Figure legends

Figure 1. During the early stage of atherosclerosis, upregulation of E-selectin, P-Selectin, and ICAM-1 induce neutrophil recruitment. Platelet-derived CCL5 activates neutrophil to release cathepsin G leading to the firm adhesion to and accumulation of neutrophils in the endothelium. ROS and proteases secreted by neutrophils activate and dysregulate endothelial cell layer and degrade the underlying extracellular matrix resulting in monocyte infiltration and LDL extravasation. Neutrophils secrete MPO that mediates LDL oxidation and promotes foam cell formation. Cathelicidin- and α-defensin-derived neutrophils activate macrophages towards pro-inflammatory state, while NETs

stimulate macrophage to release IL-6 and IL-1β that promote Th17 differentiation followed by the amplification of neutrophil recruitment. At the late stage of atherosclerosis, activated VSMCs induce neutrophil chemotaxis and release CCL7 to stimulate NETs. Histone H4-derived from NETs induce VSMC lysis and the secretion of proteases by neutrophils degrade collagen and lyse VSMCs leading to the plaque instability. Neutrophils contribute to plaque erosion thorugh NET release as well as colocalization with TLR2 of endothelial cells to induce endothelial cell stress and apoptosis.

Figure 2a. After acute myocardial infarction, ischemic/necrotic tissues release alarmins and inflammatory signals to induce neutrophil recruitments. Activated neutrophils secrete ROS, proteases, and NETs. S100A8/A9-derived from NETs induces IL-1β release following NLRP3 inflammasome priming in naïve neutrophils. IL-1β reaches the bone marrow to stimulates granulopoiesis leading tthe amplification of neutrophil production and accumulation which in turn, detriments the ischemic heart and eventual heart failure. During cardiac tissue repair, NGAL and S100A8/A9 stimulate macrophages to shift towards reparative phenotypes resulting in incresed clearance of apoptotic cells/debris . Neutrophils also secrete Annexin A1 to favor the shift of macrophage towards pro-angiogenic phenotypes that release VEGFA to promote angiogenesis in the ischemic site of cardiac tissue.

Figure 2b. Following ischemic stroke, dying neurons attract neutrophils to the ischemic area and neutrophil ROS and elastase promote endothelial cell dysfunction and vascular permeability. NETs contribute to thrombus growth thereby increasing stroke volume. Neutrophils also promote neuronal cell death that is likely mediated by NETs.

Figure 3. Periodontitis triggers systemic inflammation which cause inflammatory modulation of hematopoietic stem and progenitor cells resulting in trained myelopoiesis. In turn, as a hallmark of trained myelopoiesis, increased number of myeloid cells including neutrophils with enhanced inflammatory responsiveness may contributes to all stages of the ASCVD pathology, atherosclerosis.

Table 1. Summary of circulating molecules that are elevated in periodontitis and involved in hematopoietic tissue adaptation.

No.	Molecules	Action on hematopoietic tissue adaptation	References
1.	IL-1β	Promotes myeloid differentiation and self-renewal of HSPCs	95, 97-99
2.	TNF-α	Promotes survival of HSPCs and myeloid differentiation Induces myeloid progenitor apoptosis	100
3.	IL-6	Enhances myelopoiesis by elevating MPPs and CMPs	101
4.	Type 1 IFN	Mediates trained granulopoiesis with hyper-responsive neutrophils	102
5.	IFN-γ	Promotes HSC self-renewal and myeloid differentiation	103
6.	G-CSF	Drives granulopoiesis Promotes GMP expansion and granulocyte lineage specification	104 105

1050 **Table 2.** Summary of clinical trials

1051

No.	Name of drug	Mechanism of action	Phase	Identifier (Trial registration)	Outcome
1.	Canakinumab	Binds to IL-1β resulting in blocking the interaction between IL-1β and IL-1 receptor	Phase 3	NCT01327846	Reduction in cardiovascular events, hospitalization for heart failure, and heart failure-associated death Emergence of death due to sepsis
2.	Colchicine	Prevents microtubule formation resulting in tubulin disruption	Phase 3	NCT02551094	Reduction in ischemic cardiovascular events Increase in pneumonia
3.	Metoprolol	Attenuates neutrophil migration and infiltration by impairing the neutrophil-platelet interaction that is crucial during early phases of neutrophil recruitment	Phase 4	NCT01311700	Reduction of infarct size Improvement of cardiac function
4.	AZD5069 (a CXCR2 antagonist)	Prevents neutrophil recruitment to the site of inflammation	Phase 1 Phase 2	NCT01480739 ISRCTN48328178	No adverse events and safety concerns Currently on going
5.	AMY-101 (a complement C3 inhibitor)	Inhibits downstream activation of the anaphylatoxin C3a and C5a receptors	Phase 2a	NCT03694444	Reduction in gingival inflammation Reduction of MMP-8 and MMP-9 levels in gingival crevicular fluids

Figure 1.JPEG

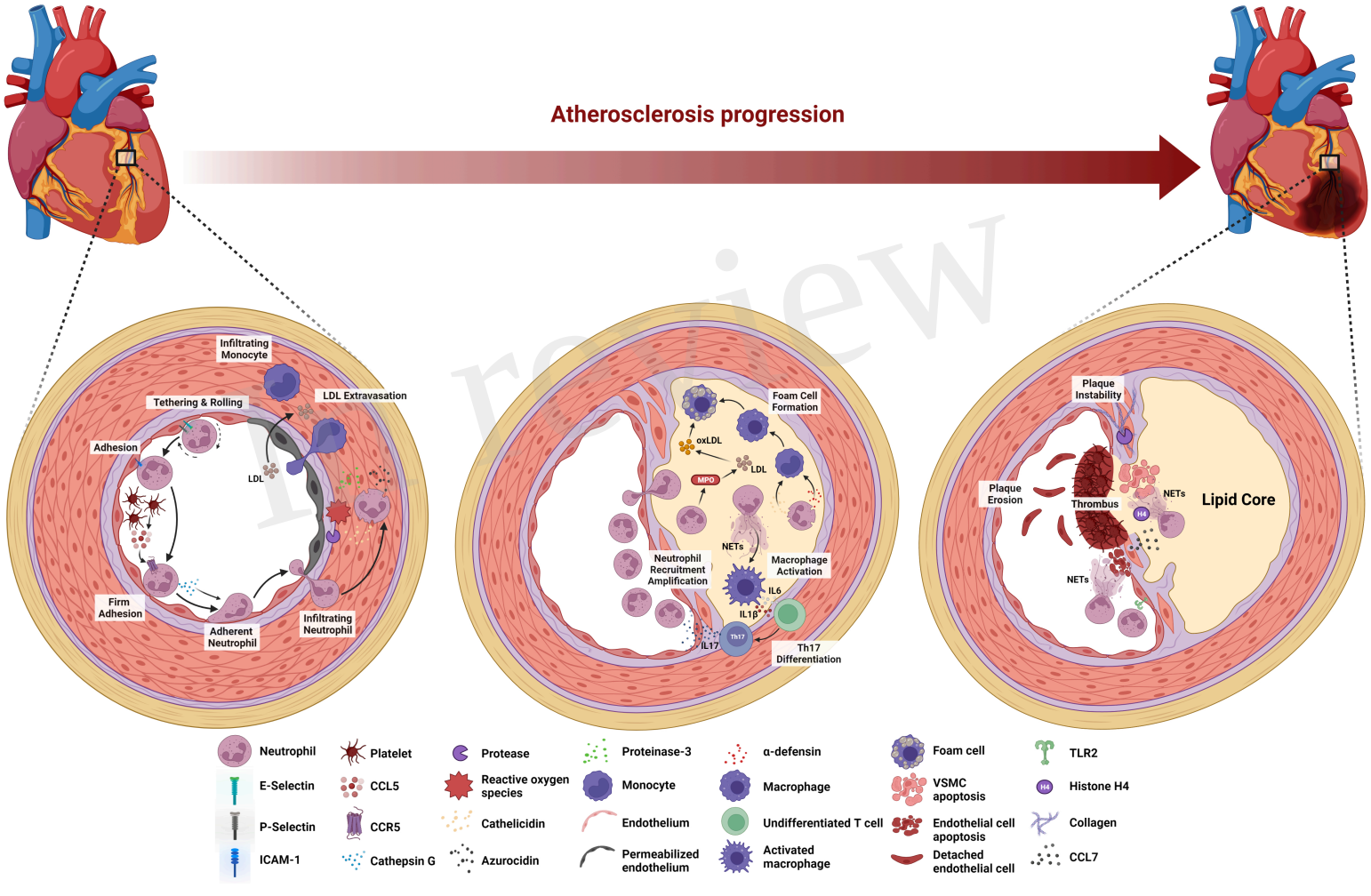

Figure 2.JPEG

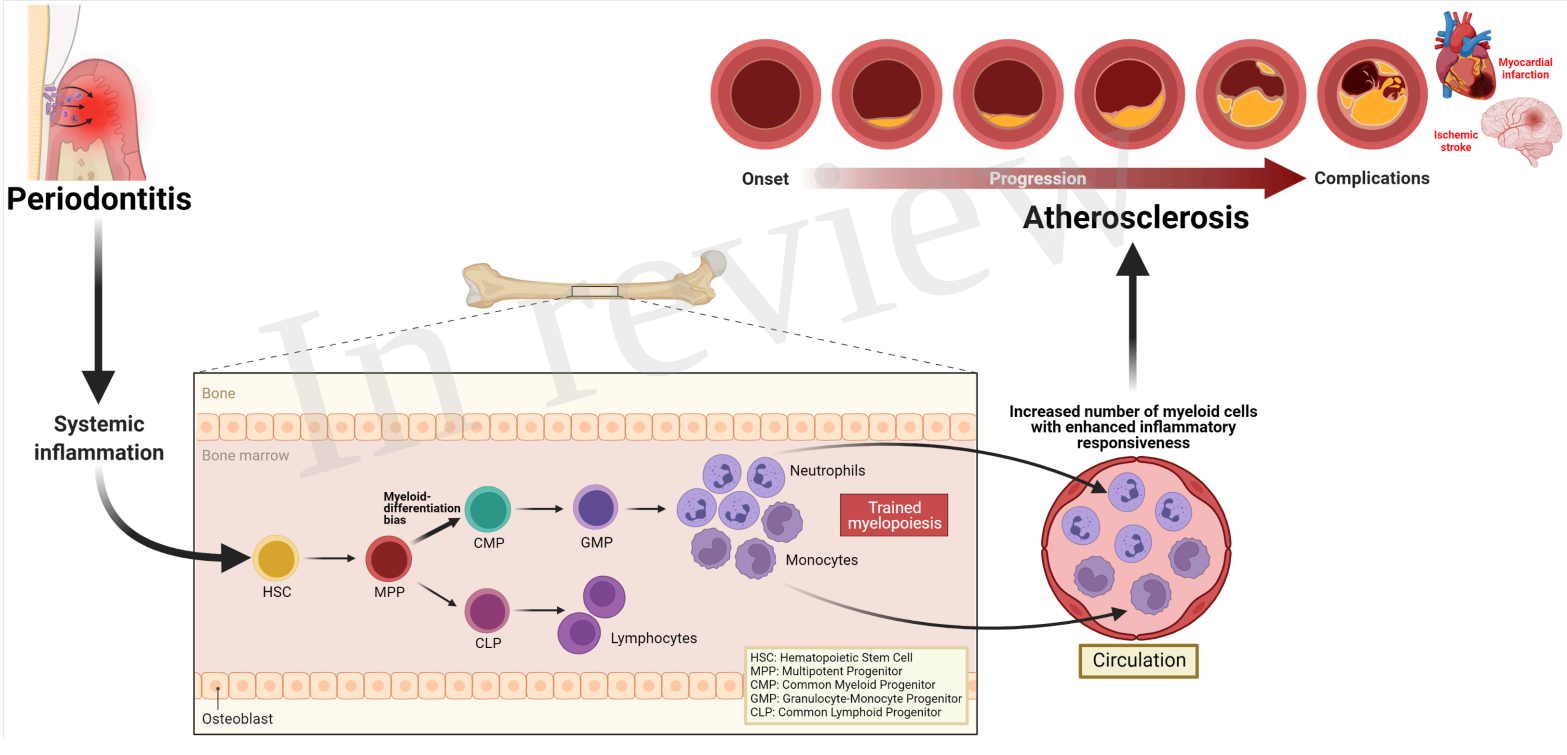

Figure 3.JPEG