J Multidisciplinary Scientific Journal

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Review

Inflammation and Peripheral Arterial Disease

Salvatore Santo Signorelli *, Elisa Marino and Salvatore Scuto

Department of Clinical and Experimental Medicine School of Medicine, University of Catania, 95122 Catania, Italy; marinoelisa@msn.com (E.M.); salvo.scuto1982@hotmail.it (S.S.)

* Correspondence: ssignore@unict.it; Tel.: +39-95-378-2545

Received: 24 January 2019; Accepted: 29 March 2019; Published: 3 April 2019

Abstract: Peripheral arterial disease (PAD) is an atherosclerotic disease closely associated with high morbidity and mortality in cardiac events. Inflammation is crucial in atherosclerosis both at triggering and in progression. Numerous inflammatory biomarkers (cytokines, matrix metalloproteinases (MMPs), selectin, intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) C-reactive protein (CRP), fibrinogen) have been measured in atherosclerotic diseases including PAD. This paper summarizes the data on the inflammatory biomarkers for PAD pathophysiology and highlights the most useful markers in monitoring PAD outcomes.

Keywords: inflammation; peripheral arterial disease; biomarkers

1. Introduction

Peripheral arterial disease (PAD) mostly affects individuals over 60 years of age; it is associated with high comorbidity and relative cardiovascular (CVD) mortality [1,2]. The prevalence of PAD is age-specific and ranges from 5% for 40 to 50 year-olds to 22.3% for 91 to 100 year-olds in the USA [3]. PAD affects up to millions of over 65 year-old patients in Europe and North America (Table 1) [4–9]. The highest prevalence of PAD was found in individuals aged over 80 years [10]. The mortality rates of PAD patients accounted for 1% to 2% of global CV deaths in 2013 [11]. Atherosclerosis lesions develop and progress with aging, representing the principal pathogenetic mechanism for PAD. Intermittent claudication, discomfort and/or pain in the lower limbs, whether exercise-related or at rest, are common diagnostic symptoms in PAD patients. These highly limit the mobility of affected subjects and impact negatively on their quality of life [12,13]. Impaired blood flow to the lower limbs is a pivotal pathophysiological concern for PAD patients.

Table 1. Prevalence of peripheral arterial disease (PAD) found in populations.

Author	Prevalence (%)	Number
Murabito, J.M. et al., 2002 [4]	3.9	5124
Selvin, E. et al., 2004 [5]	4.3	2174
Sigvant, B. et al., 2007 [6]	18	5080
Alzamora, M.T. et al., 2010 [7]	7.6	3786
Signorelli, S. et al., 2010 [8]	2.3	9100
Fowkes, F.G.R. et al., 2013 [9]	PAD prevalence increases by 28.7% in low-income or middle-income countries (LMIC) and 13.1% in high income countries.	Review of 34 published studies (2000–2010).
American Heart Association (AHA), 2018 [10]	individuals $\geq$ 80 years 22.7% individuals 40–49 years 1.6%	
Ramos, R. et al., 2009 [11]	4.5	6262

PAD is listed as one of the diseases derived from atherosclerosis, so we believe there should be a focus on the pathophysiological role played by inflammation (I) either in triggering PAD and/or to augmenting it. Ultrasound (US) tests and ankle brachial pressure index (ABI) measurements are diagnostic tools (in different settings) for PAD patients that show intermittent claudication or have continuous pain and/or severe skin damage [14]. These diagnostic tools were reproducible and reliable being both inexpensive and effective (high sensitivity, specificity) in diagnosing and monitoring PAD [15,16].

The close association between PAD and CVD risk is well known as demonstrated by several arterial co-morbidities (i.e., coronary artery disease, ischemic stroke) observed in PAD patients [14–18]. A number of chronic diseases (i.e., diabetes mellitus, dyslipidemia, and hypertension) or deleterious habits (smoking, overweight/obesity, sedentary life-style) are risk factors for PAD [19–21]. Moreover, endothelial dysfunction [22] and pro-inflammatory molecules [23,24] contribute to triggering both the damage and disarrangement of arterial walls. Consequently, these last play a key role in the pathophysiology of PAD. In recent years, the axioms about atherosclerosis as an inflammatory disease have been largely accepted [25–27] and several pro-inflammatory molecules have been studied to find out if they are agents in the pathophysiology of PAD [28,29]. It is noteworthy that the discovery of reliable inflammatory biomarkers may help to identify individuals more prone to PAD and explain CV mortality in PAD patients [30].

2. Endothelial Dysfunction

The vessels of the human body are largely maintained by endothelial barriers, which regulate a number of physiological events (i.e., immune response, fluid control, passage of electrolytes/substances). The endothelium is highly to endowed in nitric oxide generation (NO) and its release. Because NO release lowers endothelial barrier damage, endothelial damage plays a fundamental role in regulating vasodilation or vasoconstriction (known as endothelial dysfunction). Since endothelial dysfunction is widely considered an early signal of atherosclerosis without any instrument diagnosis of atherosclerotic plaque either by angiograms or ultrasound scans, reduction in NO bioactivity as well as the increased generation of oxygen free radicals (or reactive oxygen species = ROS) are effective players in endothelial dysfunction [31,32]. Large amounts of nitrites from NO oxidation are stemmed by endothelial nitric oxide synthase enzyme activity [33]. Several non-invasive methods based on the ability of endothelial cells to detect changes in shear stress have been applied to measuring endothelial dysfunction [34] which has been found in diabetes, metabolic syndrome, arterial hypertension, coronary disease, and also in individuals who smoke and are physically inactive. In PAD, endothelial dysfunction reduces the response to drug flow mediated dilatation by acetylcholine infusion [35] inducing the progression of arterial stenosis and the clinical outcomes towards critical ischemia of the lower limbs. Furthermore, there is a close inverse relationship between endothelial dysfunction and the loss of lower limb arterial pressure measured by ABI [36]. Interestingly, both lowered levels of cyclic guanosine monophosphate and urinary nitrate raised the production of endothelin-1 and plasminogen activator inhibitor-1, the support link between endothelial dysfunction and PAD [37].

3. Inflammation and PAD

Inflammation (I) is now listed as fundamental both in triggering PAD and augmenting it. It is closely related to endothelial damage and endothelial dysfunction (Figure 1). Lack of endothelium-derived nitric oxide release is the most likely symptom for inducing endothelial vasodilator dysfunction, and it is a well-recognized crucial step in atherosclerosis. Furthermore, it contributes to disarranging arterial walls, to arterial distensibility, stiffness, vasomotion, and vasomotolity [38]. Endothelial barrier damage is fundamental as a protective layer between the blood and thrombogenic subendothelial tissue [39]. The role of I in PAD pathophysiology has been progressively well established [40]. We want to summarize the results of studies on inflammatory

markers (cytokines, matrix metalloproteinase, selectin, integrines, CRP, fibrinogen) to elucidate a point of view [39–50].

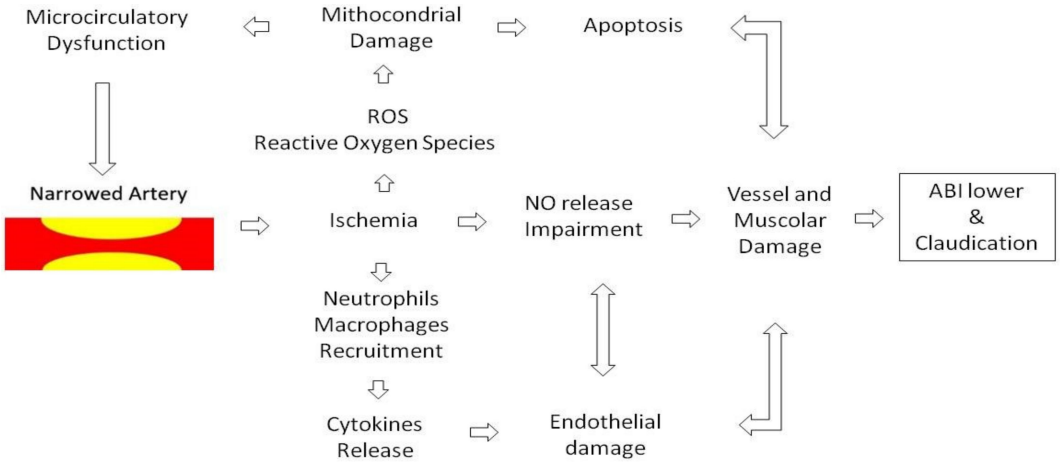

Figure 1. Overview of pathophysiological patterns in peripheral arterial disease. ABI: ankle brachial pressure index.

Regarding pro-inflammatory biomarkers, we measured the plasma concentrations of interleukin 6 (IL-6), tumor necrosis factor alfa (TNF-α), endothelial (ES), leukocitary (LS), and platelet (PS) selectins in PAD patients. Our data showed that biomarker concentrations in PAD groups were higher than those not in PAD groups (controls) and that these differences were statistically significant (p values ranged between 0.001 and 0.0001).

We measured the plasma level of neopterin (N) as a representative marker of activated macrophages. N blood concentration was higher in PADs than in controls. Interestingly, we noted that in statistical regression analysis, an inverse relationship was found between high values of N and lower ABI measurements. Additionally, we studied the effect of chronic ischemia (in PADs) on cell remodeling related to chronic ischemia. Thus, we measured matrix metalloproteinases two (MMP-2) and nine (MMP-9) concentrations in plasma drawn from PADs and controls: both were higher in PADs. We postulated a chronic hypoxia effect both in activating white blood cells and releasing inflammatory biomarkers. This data agrees with the active role played by PADs. Notably, it is known that MMPs 2 and 9 are gelatinases which interact with arterial matrices, and are released by proteinases as neutrophil elastases, and are finally released into the extracellular compartment by oxidized glutathione in oxidative stress situations. Notwithstanding our results, there is a large body of evidence from many biomarker studies (IL-1, 2, 6, 8, 10; selectins, CRP, fibrinogen) on PADs. Their focus was on the link between inflammatory biomarkers and PAD pathophysiology. Data from PAD epidemiological studies linked to the estimated frequency of cardiovascular events in PAD patients may help to focus attention on biomarkers. Unfortunately, the therapies applied to PAD patients (haemodynamic and anti-platelet drugs, with pleiotropic ability like statins) have not provided very effective results regarding peripheral arterial disease progression, nor in reducing the frequency of serious cardiovascular events. In the sections below we summarize the data from previous studies.

4. C-Reactive Protein (CRP)

C-reactive protein (CRP) is an acute-phase protein of hepatic origin [51]. Its release is stimulated by interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead cells and some types of bacteria to activate the complement system and generate pro-inflammatory cytokines [52]. CRP inhibits the release of NO, promotes the release of endothelial monocyte chemo-attractor protein-1 (MCP-1) and tissue factor. It is strongly associated with the increased risk of atherosclerotic cardiovascular disease independent of cholesterol level [53]. In contrast to the studies involving patients with coronary artery disease

(CAD), there are only a limited number of studies which have assessed CRP levels in patients with PAD. In one of these, Folsom and colleagues showed a relationship between high levels of CRP and lower ABI values [54]. In the National Health and Nutrition Examination Survey of American men and women aged ≥40 years (No. 4787), elevated levels of high sensitivity CRP, fibrinogen, and leukocyte counts were associated with lower ABI values. Fibrinogen contributes to developing atherosclerosis-promoting chemo-taxis of smooth muscle cells and monocytes/macrophages [55] and reinforcing clot structures that are difficult to lyse. The inCHIANTI study compared circulating levels of IL-6, IL-1 receptor antagonist, fibrinogen and C-reactive protein, showing raised values in PAD patients [56]. These important studies confirm that CRP is a risk factor for the development and progression of PAD.

5. Interleukin-6 (IL-6)

There is a widely accepted association between inflammation and the development of atherosclerosis, whereas there are still debates as to the helpful role played by inflammatory biomarkers. Interleukin 6 (IL-6) acts as either a pro-inflammatory or anti-inflammatory agent [57]. IL-6 is secreted by macrophages in response to pathogen-associated molecular patterns (PAMPs) and it is also secreted by several other cells including Th2 lymphocytes, B-lymphocytes, macrophages, endothelial cells and fibroblasts. IL-6 is crucial in promoting an acute inflammatory response inducing several effects:

- Expression of fibrinogen, complement and C reactive protein from hepatocytes [58,59]
- Expression of Vascular endothelial growth factor (VEGF) [60]
- Production of neutrophils in the bone marrow

Based on the aforementioned effects, IL-6 seems to play an important role in several pathophysiological processes [61–63]. Interestingly, IL-6 has been evaluated in PADs as contributing to the genesis of atherosclerosis, causing the following processes:

- Secretion of MCP-1 and IL-8 by endothelial cells and macrophages.
- Changing leukocytes into atherosclerotic plaque by producing ICAM-1 by smooth muscle cells (SMCs)
- Transformation of SMCs into foam cells

Several studies have shown that IL-6 is a reliable marker in the development of PADs [64,65]. Furthermore, studies by our working group have shown that IL-6 GG genotype contributes to the development of PADs among individuals with type 2 diabetes [66,67].

6. Interleukins in PAD

Articles have been published concerning interleukins as inflammatory biomarkers in PAD development. The biomarkers, IL-6, IL-1, IL-2, IL-8, and IL-10 have contributed to an increasing body of evidence on interleukines (ILs) in PAD, particularly concerning glycoproteins acting as pro-inflammatory agents affecting vessel walls at the triggering of atherosclerotic plaque formation and its progression.

Summarizing, the reliable role of interleukins in PAD, IL-1 contributes in PAD development [68] to extending inflammatory activity. IL-1 increases the generation of acute phase proteins, leading to cell proliferation (i.e., neutrophils). IL-1 raises chemotaxis increasing lymphocyte proliferation. It acts like endogenous pyrogens increasing the production of IL-6 which may be a major contributor to atherosclerosis in PAD patients [69].

As for IL-2 and IL-8 in atherosclerosis, their roles are still being debated. Although these ILs were enhanced in atheromatous plaques, there is a lack of evidence about the relationship between their plasma levels and PAD outcomes. Stimulated inflammatory situations are pivotal in releasing IL-8 from monocytes and macrophages. It is interesting to note the significant production of IL-8 by polymorphonuclear leukocytes [70] in PAD patients previously referred for arterial revascularization.

Furthermore, experimental data on mice negative for the IL-8 receptor (−/−) showed a limited number of atherosclerotic plaques [71,72].

IL-10 largely secreted by Th2 lymphocytes, acts as an anti-inflammatory agent. IL-10 inhibits the production of inflammatory agents by Th1 lymphocytes and macrophages. A shortfall in IL-10 has been associated to increased atherosclerotic plaque [73,74]. Interestingly, there is a direct relationship between high plasma levels of IL-10 with better prognosis in acute coronary syndrome patients [75,76]. Currently, no data is available on IL-10 in PAD.

7. Selectins

Selectin (S) is a family of three closely related glycoproteins (P-selectin expressed on platelets, on leukocytes, E-selectin expressed on endothelial cells by inflammatory cytokines stimulus, and L-selectin expressed on leukocytes, monocytes, neutrophils, and eosinophils). Selectins are structurally and functionally related to adhesion molecules which are able to bind similar carbohydrate residuals. P-Selectin is highly involved in the atherosclerotic process affecting platelet aggregation, promoting the up-regulation of tissue factor which plays a crucial role in determining arterial thrombosis [77]. Increased E-selectin plasma levels were found in PAD patients with type-2 diabetes mellitus. High E-selectin plasma levels correlate to greater activity on endothelial cells [78–80]. It is very interesting to note that in the PAD patients' protocol with controlled physical training, plasma levels of E-selectin [81] were modified.

8. Matrix Metalloproteinases (MMPs)

MMPs are a family of Zinc 2+ dependent enzymes involved in platelet aggregation, thus suggesting a possible role in the atherosclerotic process. MMPs are implicated in the proteolysis of several connective tissue proteins of the extra cellular matrix (ECM) proteins in connective tissue. ECM proteins are crucial in preserving the integrity of vessel walls and reducing MMP regulation, which simplifies monocyte invasion of the arterial wall [82]. The results of MMP studies have shown that MMP 2 and 9 might be useful in marking macrovascular damage in PAD patients and in type-2 diabetics [83]. Findings from other studies have confirmed the role of MMPs [84–86].

9. Conclusions

PAD frequently affects subjects over 60 years old for whom morbidity and mortality risk or CVD is significant. PAD is considered a marker for extended atherosclerosis. However, the efficacy of medical or intervention protocols is still debated in achieving effective targets for PAD patients. In this article, we have summarized data from research on inflammation in the pathophysiology of PAD. Several pro-inflammatory molecules were studied to elucidate any new perspectives in the pathophysiology of PAD. Different concentrations of inflammatory biomarkers were found in PAD patients, therefore suggesting that inflammatory biomarkers mark several aspects of PAD pathophysiology. In fact, biomarkers highlight activated white blood cells, endothelial dysfunction, the lack of fibrinolytic activity of the endothelium membrane, and consequently, the occurrence of a pro-coagulative status. These dysfunctions are effective in worsening the chronic ischemia of PAD patients to more severe or critical ischemia of the peripheral arteries. Additionally, the pathophysiological role played by inflammation can lead to novel approaches both in screening individuals more prone to atherosclerosis and in managing PAD treatment. We point out that these are interesting objectives particularly considering that vasoactive drugs usually given in PAD treatment appear not to fulfill all the therapeutic targets. More research is needed on the old and new inflammatory biomarkers in PAD to improve the database on their mechanisms and on novel effective therapies.

Author Contributions: Conceptualization, S.S.S.; Methodology, S.S.S., S.S., E.M.; Formal analysis, S.S.S., S.S., E.M.; Data curation, S.S.S.; Writing—original draft preparation, S.S.S., S.S., E.M.; Writing—review and editing, S.S.S., S.S., E.M.; Supervision, S.S.S.

Funding: This research received no external funding.

Conflicts 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.

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