Peripheral artery disease (PAD), which consists of partial or complete obstruction of the arteries in the lower limbs, is one of the most common manifestations of atherosclerosis, affecting ≈27 million individuals in Europe and North America. Its main symptomatic expression, intermittent claudication, was first described by the French veterinarian Bouley in a horse affected by progressive limping and lameness consequent to a fibrous clot that occluded the femoral arteries of the posterior limbs. In humans, this condition was noted by Brodie in 1846, but it was Charcot who in 1858 clearly defined and described the syndrome (and used the term "intermittent claudication"). Reproducibly elicited by walking-induced muscle ischemia and consistently relieved by rest that allows reperfusion of the affected limb, intermittent claudication may be considered "leg effort angina." Indeed, for a long time, treatment was aimed exclusively at relieving leg symptoms and improving the functional status of affected individuals. However, in the 1950s, Stammers and Allen et al independently observed that patients with claudication were at high mortality risk. Subsequent prospective studies confirmed that patients with PAD rarely progress to limb loss but that the presence of PAD is a powerful and independent predictor of cardiac and cerebral ischemic events. However, this increased risk appears to be poorly related to classic risk factors, suggesting that once PAD is established, subsequent cardiovascular risk is related to the severity and extent of the underlying atherosclerotic disease and possibly other factors.

It is well established that hypertension, smoking, diabetes mellitus, and hypercholesterolemia play a major role in the initiation and development of atherosclerosis and its clinical manifestations, although the prognostic potency of each of these factors in atherogenesis differs in the various arterial beds. As early as 1815, the year that cholesterol was discovered although not yet correlated to atherosclerosis, the London surgeon Joseph Hodgson published a monograph on vascular disease in which he cited inflammation as the underlying cause of atherosclerosis. In 1858, the German pathologist Rudolf Virchow found inflammatory cells in vascular plaques, but it was Sir William Osler who, in 1908, implicated inflammation and infection in the pathogenesis of atherosclerosis. However, the inflammation hypothesis was ignored for nearly a century, during which time atherosclerosis was firmly believed to be a cholesterol disease. The causative role of inflammation in the atherogenic process was established only at the end of the last millennium when many lines of evidence suggested alternative mechanisms to the cholesterol theory, and Russell Ross branded atherosclerosis an inflammatory disease. In actual fact, atherosclerosis is not simply a disorder of pathological lipid deposition but is regarded as a dynamic and progressive pathophysiological process arising from a combination of endothelial dysfunction and inflammation interacting with the standard risk factors that contribute to the initiation, clinical manifestations, and cardiovascular risk of all atherosclerotic diseases.

Endothelial dysfunction in PAD has been discussed previously. The present review is devoted to the role played by inflammation in PAD, summarizing the data showing that increased levels of inflammatory markers are associated with the development of PAD, its cardiovascular comorbidity, and risk of developing cardiac and cerebrovascular ischemic events. The final part of the review describes the inflammatory mechanisms presumed to contribute to claudication and its severity.

Inflammation and Risk of Developing PAD

Inflammation is important for the initiation and progression of PAD, and the inflammatory mediators involved in this process are similar to those contributing to the development of coronary artery disease (CAD). There are several candidate inflammatory triggers, including traditional risk factors that exert a proatherogenic role, at least in part, through an inflammatory mechanism. Cigarette smoking and diabetes mellitus, the strongest predictors of developing PAD, promote oxidative stress, which directly and indirectly enhances inflammatory pathways. Immune cell adhesion molecule-1 from endothelial cells. Angiotensin II increases the expression of proinflammatory cytokines such as interleukin-6 and monocyte chemoattractant protein-1 by arterial smooth muscle cells. In addition, dyslipidemia may activate inflammatory functions by modifying the oxidation of low-density lipoproteins and of very low-density lipoproteins. However, unlike in CAD, dyslipidemia has...
relatively less importance in predicting the risk of lower-extremity occlusive disease.\(^{41}\) Other conditions that imply a systemic inflammatory response and may predispose the arterial vessels of the lower limbs to atherosclerosis are single-nucleotide polymorphisms in genes encoding inflammatory molecules, infections, and nonvascular systemic diseases such as rheumatoid arthritis and systemic lupus erythematosus.

Whatever the inflammatory trigger, a large number of cross-sectional and longitudinal studies demonstrate a close link between inflammation and PAD.\(^{19,27}\) Noteworthy, each of the inflammatory molecules investigated in those studies is not simply a marker of inflammation but plays an active role in peripheral atherogenesis.\(^{42–48}\)

In 1998, Ridker et al., in a prospective, nested case-control study carried out in apparently healthy men enrolled in the Physician’s Health Study, found that the relative risk of developing PAD increased significantly with each increasing quartile of baseline C-reactive protein (CRP) concentration, such that men in the highest quartile (>2.1 mg/L) had a 2-fold increased risk compared with men in the lowest quartile (<0.55 mg/L). Notably, this result was independent of body mass index, hypercholesterolemia, diabetes mellitus, and a family history of premature atherosclerosis. The relationship between CRP and PAD risk has also been demonstrated in young healthy women.\(^{20}\) The Physician’s Health Study also showed that elevated levels of soluble intercellular adhesion molecule-1, but not of soluble vascular cell adhesion molecule-1, are independently associated with the development of PAD.\(^{21}\) In analyses adjusted for age and smoking (2 major risk factors for PAD), the odds ratio in the highest compared with the lowest quartile of soluble intercellular adhesion molecule-1 was 3.9 (95% confidence interval [CI], 1.7 to 8.6). Importantly, elevated soluble intercellular adhesion molecule-1 remained significantly associated with the development of PAD after additional adjustment for lipid and nonlipid factors, including CRP. This finding, in association with the fact that combination of elevated levels of both biomarkers appeared to identify individuals at the greatest risk, suggests that CRP and intercellular adhesion molecule-1 play independent roles in the development of peripheral atherosclerosis.

The Edinburgh Artery Study produced results similar to those of the Physician’s Health Study.\(^{19,27}\) During a follow-up of 17 years, 209 of 1519 subjects (14%) developed PAD. Of these, most (ie, 169) were diagnosed as having intermittent claudication. After correction for age and sex, a number of inflammatory molecules, among which were CRP, interleukin-6, soluble intercellular adhesion molecule-1, and lipoprotein(a), were found to be associated with increased PAD risk. However, at variance with the Physician’s Health Study, the hazard ratio for PAD of soluble intercellular adhesion molecule-1 was attenuated after adjustment for classic risk factors. This between-study discrepancy is probably due to differences in experimental design and statistical methods. Thus, there is a strong signal that systemic inflammation plays a role in peripheral atherosclerosis over and above the traditional risk factors.

### Genetics, Inflammation, and PAD

Some family studies have assessed the heritability of PAD.\(^{49–51}\) In the National Heart, Lung, and Blood Institute Twin Study in which 8.2% of the population had PAD, concordance rates for twin-pair similarity for low ankle-brachial index (ABI) were 33% for monozygotic pairs and 31% for dizygotic pairs.\(^{49}\) These findings indicate that the twin of a participant with PAD was 4 times more likely to have PAD than a randomly selected individual. Results suggested that genetic factors determined \(\approx48\)% of the variability in ABI after adjustment for cardiovascular disease risk factors. A subsequent study assessed the heritability of low ABI among participants in the Framingham Offspring Study.\(^{50}\) Overall, genetic determinants contributed to 21% of the variability in the ABI, a modest heritable effect, and cardiovascular disease risk factors contributed to 14% of the variability. Results also indicated that the majority of the interindividual variability in the ABI could not be explained by genetic or environmental determinants. In the Genetic Epidemiology Network of Arteriopathy study, after adjustment for cardiovascular disease risk factors, heritability for the ABI was 19.5% for blacks and 21.2% for non-Hispanic whites.\(^{51}\) Together, these studies suggest a moderate, significant heritability for PAD. Unfortunately, to date, no definitive genetic markers have been identified for PAD. Genetic determinants of PAD may reveal proteins implicated in the pathophysiology of lower-extremity atherosclerosis, thereby identifying mechanisms for the development and progression of lower-extremity atherosclerosis. In this context, the finding that a polymorphism of the EE genotype of intercellular adhesion molecule-1 significantly increases the risk of PAD reinforces the concept that inflammation plays a relevant role in PAD development.\(^{52}\) Furthermore, Flex et al.\(^{53}\) studied 157 PAD patients and 206 control subjects and found that gene polymorphisms not only of intercellular adhesion molecule-1 but also of interleukin-6, E-selectin, monocyte chemoattractant protein-1, and matrix metalloproteinases 1 and 3 were independently associated with PAD. Conversely, distribution of CRP genotypes did not differ significantly between patients and control subjects. Although that study had several limitations (It is a relatively small case-control study and thus recruitment bias may not be excluded, and the study population was relatively small and is not representative of all PAD patient groups), it provides a rationale for the association between many inflammatory molecules and PAD risk. Indeed, plasma levels and/or functional activity of these inflammation determinants may be influenced by functional single-nucleotide polymorphisms of the corresponding genes. However, these are small studies of single candidate genes that are probably underpowered to detect independent relationships between any particular gene polymorphism and PAD.

### Infection and PAD Risk

The importance of inflammation in atherogenesis prompted the idea that an infectious agent could be the link between chronic inflammation and PAD. The most compelling evidence comes from seroepidemiological studies of 3 pathogens: *Chlamydia pneumoniae*, *Helicobacter pylori*, and *Cytomegalovirus*. To examine the relations between infection,
inflammation, and occurrence of PAD. Bloemenkamp et al.\textsuperscript{54} in a multicenter, population-based, case-control study, measured IgG antibody titers and CRP levels in 228 young women affected by PAD and 643 control women. After adjustment for potential confounders, the odds ratios for PAD women with serological evidence of infection with \textit{C pneumoniae}, \textit{Helicobacter pylori}, and \textit{Cytomegalovirus} were 2.0 (95\% CI, 1.3 to 3.1), 1.6 (95\% CI, 1.1 to 2.2), and 1.6 (95\% CI, 1.1 to 2.3), respectively. In addition, the cumulative number of infections was positively related to the risk of PAD, and this relationship was stronger in women with a high CRP level than in those with a low CRP level. This suggests that an inflammatory response might indeed be involved in the process that relates infection with PAD. In a small study, anti-\textit{C pneumoniae} IgA titers and CRP levels were higher in PAD patients than in control subjects.\textsuperscript{55} On multivariate analysis, only smoking, CRP, and anti-\textit{C pneumoniae} IgA remained independently associated with the disease. More intriguing are the results of the Bruneck study, a population-based study of the epidemiology and pathogenesis of atherosclerosis and arterial disease.\textsuperscript{56} In a large random population of 1000 subjects, antibodies to \textit{C pneumoniae}, but not \textit{H pylori} and \textit{Cytomegalovirus}, were weakly but significantly associated with PAD (odds ratio 1.09; 95\% CI, 1.00 to 1.18). The association was attenuated (odds ratio 1.07; 95\% CI, 0.98 to 1.18) when some potential confounders were included in the multivariate model. However, when femoral intima-media thickness was used as an outcome variable instead of femoral atherosclerosis, \textit{C pneumoniae} remained a significant risk factor for PAD in multivariate analysis.

Some caveats have a bearing on the results of these seroepidemiological studies. First, several factors can confound the results.\textsuperscript{57} For example, smokers may have a higher incidence of \textit{C pneumoniae}-induced bronchitis. Therefore, evidence of infection with \textit{C pneumoniae} may merely be a marker of tobacco use, which is one of the main risk factors for PAD development.\textsuperscript{29} Second, there is a publication bias against studies with negative findings. Finally, atherosclerosis is very common in developed countries, and many adults have serological evidence of prior infections with \textit{Cytomegalovirus} and \textit{C pneumoniae}. It is difficult to distinguish coincidence from causality when the majority of the population studied has evidence of both infection and atherosclerosis.

Nonetheless, support for the infection theory comes from studies showing an association between poor oral health and PAD. The Veterans Affairs Dental Longitudinal Study showed that of 1030 subjects followed up for >25 to 30 years, those with clinically significant periodontal disease at baseline had a 2.27 (95\% CI, 1.3 to 3.90) increased risk of developing PAD.\textsuperscript{58} More recently, the Health Professionals Follow-Up Study demonstrated that the risk of PAD was significantly higher in men with a history of periodontal disease or with any tooth loss occurring during follow-up than men without periodontitis or without any tooth loss. Notably, the association was present between cumulative tooth loss and PAD (relative risk, 1.39; 95\% CI, 1.07 to 1.82) but not between PAD and tooth loss in the 2 years before the end of the follow-up.\textsuperscript{59} Because the most common cause of tooth loss in older adults is periodontal disease, these findings suggest that 2 years may be too short for oral infection/inflammation to have an impact on PAD. Obviously, the infection theory would gain support should antibiotic therapy be found to reduce the incidence of PAD, but results in this regard are inconclusive.\textsuperscript{60-63} However, a recent publication evaluated the effect of a potent anti-chlamydial antibiotic on treating claudication in PAD, and the trial failed to demonstrate any clinical benefit on treadmill exercise performance or cardiovascular events.\textsuperscript{64}

**Autoimmune Disease and PAD Risk**

Additional evidence that inflammation contributes to PAD risk is that many patients with rheumatoid arthritis or systemic lupus erythematosus develop atherosclerosis in the arteries of the lower limbs.\textsuperscript{65-69} Indeed, despite a clear distinction in their pathophysiology, rheumatoid arthritis and systemic lupus erythematosus share an inflammatory process that is strikingly similar to the process leading to atherosclerosis.\textsuperscript{70} Unfortunately, however, most studies investigating the association of rheumatoid arthritis and systemic lupus erythematosus with PAD are small and cross-sectional.\textsuperscript{66-69} An exception is the study by Liang et al.\textsuperscript{65} In this retrospective medical record review of 609 patients with incident rheumatoid arthritis diagnosed during 1955 to 1994, patients were followed up from 1955 to 2000 (median, 11.8 years) for incident noncardiac vascular disease. During follow-up, 68 patients (11\%) developed PAD, with a 30-year cumulative incidence of 16.1\%. A Cox analysis adjusted for age, sex, body mass index, smoking, and rheumatoid factor showed that rheumatoid arthritis was a significant predictor of PAD (hazard ratio, 2.29; 95\% CI, 1.20 to 4.34). Interestingly, rheumatoid arthritis was not associated with the occurrence of cerebrovascular events (hazard ratio, 1.52; 95\% CI, 0.72 to 3.21). In conclusion, several lines of evidence indicate that inflammation plays a pivotal role in the development of PAD.

**Inflammation and Cardiovascular Comorbidity**

Because atherosclerosis is a systemic disease, patients with manifest atherosclerosis in 1 vascular territory may have atheromatous plaques, although asymptomatic, in other arterial territories. Therefore, it is important to understand the epidemiology and pathogenesis that predispose to the coexistence of various manifestations of the disease, as well as the implications of multiple vascular bed involvement. Intriguingly, in the Reduction of Atherothrombosis for Continued Health (REACH) registry, a concomitant affected arterial territory was present in one quarter of patients with clinically manifested CAD, one third of patients with cerebrovascular disease, and more than half of the PAD patients.\textsuperscript{71} Given the systemic nature of atherosclerosis and the fact that its various clinical manifestations share the same risk factors, one would expect a more homogeneous distribution of vascular comorbidity. Instead, as indicated above, the prevalence of comorbid conditions seems to be related to the vascular population screened. Furthermore, in the entire population of the REACH registry (n=67 888), the 1-year atherothrombotic event rates (cardiovascular death, myocardial infarction, stroke, or hospitalization for a cardiovascular event) were...
Inflammation and Carotid Artery Disease

Several clinical and histopathological studies indicate that the severity of carotid artery disease (a manifestation of peripheral atherosclerosis) is related to inflammation. The inflammatory infiltrate of carotid plaques was more pronounced in patients with than in those without cerebral ischemic symptoms. Furthermore, the extent of the systemic inflammatory status has been found to parallel the degree of carotid stenosis.

However, inflammation is related not only to the degree of plaque stenosis but also to plaque morphology, which plays a distinct pathophysiological role in the development of stroke. In fact, many cerebrovascular events are associated with carotid stenoses <75%, thereby implicating other mechanisms in these events (eg, cardiac or aortic embolism) with embolism from the carotid bifurcation as the most frequent pathogenetic mechanism of cerebral ischemia. Histopathological data have led to the concept that plaques with a soft lipid-rich core, a thin cap, and inflammation in cap and shoulder are unstable and prone to rupture. This type of plaque is identifiable at B-mode ultrasound by its low echogenicity. In contrast, plaques that consist mainly of fibrin and collagen, and thus are more stable, present high echogenicity.

Although PAD patients have a marked inflammatory status, and despite the compelling evidence of a strict relationship between inflammation and carotid vascular disease severity, this association has been poorly investigated in PAD. We previously demonstrated that hypoechoic, presumably inflamed plaques in the femoral arterial bed were related to hypoechoic plaques in the carotid arteries. Specifically, hypoechoic carotid plaques were identified in 55.8% patients with hypoechoic femoral plaques and in only 32.0% with echo-rich femoral plaques (P < 0.001). In a multivariate analysis adjusted for age, sex, and traditional risk factors, femoral hypoechoic plaque was the only significant predictor of the presence of hypoechoic carotid plaques (odds ratio, 3.87; 95% CI, 1.53 to 9.83). In our study and others, carotid plaque echolucency was strongly associated with neutrophil and leukocyte count. This is consistent with the finding that, in patients with acute myocardial infarction, the leukocyte number increased proportionally with the number of carotid plaques defined as unstable at B-mode ultrasound. Similarly, Lombardo et al reported that, in CAD, CRP levels were higher in patients with complex unstable carotid plaques than in patients with stable plaques. Collectively, these findings seem to confirm that "plaque instability" is a polyvascular phenomenon closely linked to inflammation.

Inflammation, PAD, and CAD

The prevalence of CAD in PAD is very high, ranging between 43% and 90%, depending on the sensitivity of the technique used to detect CAD. In contrast, the prevalence of PAD in CAD patients is generally reported to be <25%. This suggests that PAD and CAD differ with respect to the type and/or intensity of the mechanisms favoring the propagation of atherosclerosis to other vascular territories. This could be related to the fact that PAD patients could be genetically more prone to develop atherosclerosis than patients with CAD. In this regard, it is of interest to note that when PAD and CAD coexisted in the 838 patients of the Program on Surgical Control of Hyperlipidemias (POSCH) study and in the 1712 individuals of the 2 Italian cohorts of the Seven Countries Study of Cardiovascular Disease, the disease developed earlier in the peripheral than in the coronary bed. This may be related to the fact that CAD patients are treated more aggressively to lower the risk of future cardiovascular events than PAD patients, who are largely untreated in terms of cardiovascular risk reduction. Therefore the PAD population remains exposed to a very high risk of developing atherosclerosis in other vascular districts. Another hypothesis is that the large vascular bed of the lower limbs, where inflamed plaques are common, may release inflammatory mediators that contribute to the development of CAD. Indeed, we recently observed that human coronary endothelial cells, incubated with serum of venous femoral blood from the affected leg, released more monocyte chemoattractant protein-1 than when they were incubated with serum of the aorta of the same PAD patients. This difference disappeared when the cells were incubated with serum withdrawn from healthy legs of control subjects. These in vitro results, which indicate the presence of inflammatory triggers in the venous blood leaving the affected leg, substantiate data obtained in humans. In fact, in CAD plus PAD patients, a higher transfemoral gradient of neutrophil myeloperoxidase content (an index of neutrophil activation and a well-established marker of inflammation) correlated with coronary artery endothelial function (r = 0.59, P < 0.05). This relationship was much greater after maximally tolerated exercise (r = 0.79), which increased neutrophil activation across the affected circulation, as indicated by the increase in myeloperoxidase transfemoral concentration. Thus, patients who had the greatest inflammatory response in the claudicating limb with exercise showed the greatest coronary artery endothelial dysfunction. These data are in line with previous studies showing that, in PAD patients, ischemic exercise promotes neutrophil activation and is associated with increased endothelial permeability at distant sites.

Inflammation may also contribute to the pathophysiological and clinical implications of the presence of PAD in CAD. As reported above, the prevalence of PAD is relatively low in CAD, but its presence has an important clinical relevance because it entails more widespread and severe coronary atherosclerosis and a greater prevalence of previous myocardial infarction. In effect, the cardiovascular risk profile is more pronounced in CAD plus PAD patients than in CAD-alone patients. However, the more severe coronary atherosclerosis and worse natural history in CAD plus PAD appear to be independently associated with increased markers of inflammation such as CRP, serum amyloid A, interleukin-6, and neopterin rather than with...
classic risk factors. What remains to be determined is whether and to what extent the increased levels of inflammatory molecules in CAD plus PAD result from a primary “extravascular” activation of the acute-phase response or whether they originate from the site of the active plaques in the lower limbs; both mechanisms are possible. However, the above-reported association of a high inflammatory status of the affected leg with coronary endothelial dysfunction, which is a key factor in atherosclerosis progression, may be a link between PAD and CAD severity. Indeed, we previously demonstrated that the severity of coronary atherosclerosis was related to the degree of inflammatory response in the affected PAD limb. This suggests that it is not PAD itself but its systemic inflammatory activity that is associated with a greater number of coronary stenoses, a higher prevalence of 3-vessel CAD, and a higher rate of previous myocardial infarction.

Notably, in CAD patients, the coexistence of PAD also entails more severe carotid vascular disease. Compared with the CAD-alone group, the CAD plus PAD group included fewer subjects without carotid plaques (22.2% versus 10.1%; \( P = 0.035 \)) and had more hypoechoic carotid plaques and symptomatic plaques. Inflammatory status, measured as leukocyte number, was more pronounced in CAD plus PAD than in CAD-alone patients. With multivariate analysis, the strongest predictor of hypoechoic carotid plaques was leukocyte count (odds ratio, 6.70; 95% CI, 2.13 to 21.10; \( P = 0.001 \)), followed by the coexistence of PAD and CAD (odds ratio, 4.20; 95% CI, 1.45 to 12.14; \( P = 0.008 \)). Therefore, as previously suggested, the greater number of hypoechoic, presumably unstable, carotid plaques in CAD plus PAD patients may be related to the more severe inflammatory profile in these patients compared with CAD-alone patients.

In conclusion, increased inflammation may help explain why the prevalence of clinically manifested CAD in PAD is much higher than the prevalence of PAD in CAD and why the coexistence of PAD in CAD patients portends more severe coronary and carotid atherosclerosis. The intriguing hypothesis that active plaques in the lower-limb arteries could play a causative role in the development and evolution of atherosclerosis in other vascular districts needs to be verified in large prospective studies.

PAD, Inflammation, and Cardiovascular Risk

PAD patients are exposed to a very high cardiovascular risk, which, in some studies, was found to be even greater than in isolated CAD or cerebrovascular disease patients. In PAD patients, PAD was found to be a stronger predictor for cardiovascular death and total mortality than prior myocardial infarction. However, unlike CAD and cerebrovascular disease, the increased cardiovascular risk of PAD patients is poorly influenced by classic risk factors and previous cardiac or cerebral ischemic events. As discussed above, the major prognostic indicator is PAD severity evaluated by ABI or clinical staging, but a large body of evidence suggests that inflammation may independently affect the susceptibility of PAD patients to future cardiovascular events.

Indeed, several inflammatory markers play a predictive role in PAD. The best studied is CRP, and the American Heart Association has endorsed its use as an independent marker of increased risk of cardiovascular events. In 51 patients who underwent lower-limb revascularization for severe intermittent claudication or critical leg ischemia, elevated CRP levels (>9 mg/L) were associated with an increased risk of myocardial infarction after adjustment for the Eagle score index and previous CAD at 2 years of follow-up. Notably, CRP was significantly associated with both all-cause and cardiovascular mortality among patients who died during the 2 years after CRP assessment, but the biomarker lost its prognostic value for deaths occurring between 2 and 3 years after assessment, although CRP levels remained high. They obtained similar results for serum amyloid A. However, Vidula and coworkers did not compare the predictive power of CRP when combined with classic risk factors or ABI. A recent study by Schlager et al showed that in 447 patients who underwent peripheral angioplasties because of symptomatic PAD, baseline CRP levels were significant predictors of major cardiovascular events at follow-up after adjustment for multiple confounding factors except ABI.

Despite these positive associations, the predictive role of CRP has been reassessed in recent years. In particular, in 2 recent studies, CRP was poorly associated with an adverse outcome in PAD. Hogh et al prospectively studied 452 patients with symptomatic PAD and concluded that, although CRP was associated with future arterial events, it cannot stand alone as a predictive tool. In another study, we assessed the predictive value of CRP and myeloperoxidase in 156 patients with intermittent claudication followed up for a median period of 17.5 months. Receiver-operating curve analysis revealed that CRP did not significantly contribute to predict future myocardial infarction or stroke, with the C statistic being only 0.53 (95% CI, 0.41 to 0.65; \( P = 0.670 \)). Cox analysis corrected for classic risk factors, body mass index, ABI, concomitant coronary and cerebrovascular disease, and myeloperoxidase levels confirmed that CRP was...
not an independent predictor of outcome (hazard ratio, 0.88; 95% CI, 0.60 to 1.29; P = 0.514). Noteworthy, CRP failed to predict outcome even when we pooled the patients with CRP >1 mg/L, i.e., patients at medium and high risk according to the American Heart Association guidelines, and compared their risk to those with baseline CRP values <1 mg/L.\(^{148}\) Thus, in interpreting the independent contribution of CRP on cardiovascular risk in PAD, it is necessary to have long-term follow-up and to incorporate the traditional risk factors and measures of PAD disease severity (eg, ABI) into the model. Under those conditions, CRP may be less clinically important relative to the underlying disease severity.

Other inflammatory markers may play a role in the natural history of PAD. For example, receiver-operating curve analysis showed that myeloperoxidase had a significant discrimination capability (C statistic, 0.69; 95% CI, 0.61 to 0.75; \(P = 0.015\)), and an elevated serum myeloperoxidase level was associated with a 6.80-fold (95% CI, 1.20 to 38.69; \(P = 0.031\)) increased risk of future myocardial infarction or stroke compared with lower levels.\(^{142}\) Notably, the addition of myeloperoxidase measurement to ABI improved the ability to identify patients at very high risk.\(^{142}\)

Inflammatory biomarkers that are likely independently associated with increased vascular risk in PAD are adhesion molecules and leukocyte count.\(^{143–147}\) Increased levels of soluble intercellular adhesion molecule-1 significantly predict adverse cardiovascular events in patients with ultrasound-proven atherosclerosis (stenosis >70%) of the carotid, iliac, or femoral artery.\(^{143}\) In another study of a well-defined population with intermittent claudication, increased plasma levels of soluble vascular cell adhesion molecule-1 were associated with a 4-fold increased cardiovascular risk.\(^{144}\) The predictive value of soluble vascular cell adhesion molecule-1 was independent of classic risk factors, previous myocardial infarction, and stroke and added to the predictive value of ABI.

Elevated leukocyte count has been widely shown to be a negative prognostic indicator in healthy subjects and in patients with CAD.\(^{154–157}\) Conversely, in PAD, only a few studies investigated the predictive value of this risk marker. The Prevention of Atherosclerotic Risk Complication by Ketanserin study, which included 1969 claudicants, showed that baseline leukocyte count was a significant predictor of myocardial infarction, stroke, and vascular death, but the relative hazard supporting this association was not reported.\(^{145}\) In another study, 2111 PAD patients were followed up for 18 months, and elevated total leukocyte count was significantly associated with a higher incidence of composite cardiac, cerebral, and peripheral events.\(^{146}\) Haumer et al\(^{147}\) showed that an elevated neutrophil count, but not total leukocyte count, was associated with the risk of developing a composite cardiovascular end point (myocardial infarction, percutaneous coronary interventions, coronary artery bypass grafting, stroke, carotid revascularization, and death). However, this study has several weaknesses, including the facts that the PAD population was confounded by including more severe patients in whom local infection could have increased the white blood cell count and that the analysis was not adjusted for ABI. Recently, in a homogeneous population of 259 patients with intermittent claudication, our group observed that both elevated total leukocyte count and neutrophil count predicted the incidence of myocardial infarction and stroke and added to the prognostic value of ABI.\(^{158}\)

In conclusion, circulating acute-phase reactants elicited by vascular inflammation mark an increased risk for vascular events in PAD. Therefore, the amount of atherosclerosis (ABI) and activity of atherosclerosis (inflammation) may represent independent characterizations of the disease risk, each with its own measurement and implications. This probably explains why the addition of inflammatory markers to ABI generally improves risk stratification in PAD.\(^{138,142,144,158}\) However, the true association of systemic inflammation with cardiovascular risk needs to be confirmed in PAD with studies that include a sufficiently large population followed up longitudinally, including measures of the above inflammatory markers, traditional clinical risk factors, and measures of PAD disease severity, to truly define the independent contribution of inflammation on PAD natural history.

### Pathophysiology of Intermittent Claudication: Historical Theories

Patients often describe claudication pain as episodic, which may be accompanied by physical findings of foot blanching and disappearance of pedal pulses. These are typical features of vasoconstriction; thus, Erb,\(^{159}\) who in 1898 attributed muscle pain to reduced blood supply, suggested that claudication involved a functional vasospastic factor in addition to organic changes. In 1922, Comroe\(^{160}\) demonstrated that foot pulses disappear in some patients with claudication during exercise and return on resting. He believed that this phenomenon was purely vasospastic in origin and thus called it “paroxysmal angiospasm dolorosa.” The theory that vasospasm could contribute to claudication persisted for a long time. During the 1940s, several investigators reported that, contrary to healthy individuals, claudicants had diminished oscillographic curves after exercise.\(^{161–163}\) This was attributed to constriction of the muscle arteries caused by the release of abnormal substances instead of the normal vasodilating metabolites that are produced during nonischemic exercise.\(^{162}\)

In 1931, Lewis et al\(^{164}\) reported that hyperemia in a vascular district may be accompanied by simultaneous shunting of blood from other vascular territories. This phenomenon may be useful under particular circumstances because it allows rational use of circulating blood volume without relevant changes in overall cardiac output and arterial pressure. In contrast, this hemodynamic mechanism, also known as the “blood-steal phenomenon,” may be harmful in claudicants by shunting exercise blood flow from ischemic toward normoperfused areas.\(^{165,166}\) From these observations, the contribution of abnormal blood flow in large to medium conduit arteries contributing to intermittent claudication was suggested. However, the central role of a reduced blood flow and oxygen delivery relative to skeletal muscle demand is now well established as a major component of the pathophysiology of claudication.\(^{167,168}\) Whether additional vasoconstriction contributes to claudication over and above the reduced
blood flow caused by the large-vessel occlusions has been suggested but not been well studied.\textsuperscript{169}

Subsequently, the interest of investigators shifted from “vessel” to “blood.” Indeed, Dormandy et al\textsuperscript{170} reported that PAD patients have a higher blood viscosity than age-matched control subjects and concluded that this abnormality, according to the Pousseille law, could be a critical factor for exercise-induced ischemia. In 1976, Reid et al\textsuperscript{171} observed that red cell flexibility is reduced in patients with intermittent claudication, and thus the passage of erythrocytes through nutritive capillaries might be compromised by a microcirculatory vessel plugging.

**Inflammation as an Acute Response to Exercise in Patients With Claudication**

Although the pathophysiology of intermittent claudication is attributed primarily to a flow-limiting stenosis or occlusion of a conduit artery that limits oxygen delivery during exercise, a large body of evidence indicates that, with exercise, limb ischemia evokes an acute systemic response characterized by increased oxidative stress, inflammation, and endothelial dysfunction.\textsuperscript{118,119,121} In affected individuals, exercise induces an increase in plasma levels of thiobarbituric acid–reactive substances, accompanied by an increase in thromboxane and interleukin-8 and elevated plasma levels of soluble intercellular adhesion molecule-1, vascular cell adhesion molecule-1, von Willebrand factor, E-selectin, and thrombomodulin.\textsuperscript{118,119,172–175}

These observations suggest an acute inflammatory response to muscle ischemia during exercise (and possibly reflecting reperfusion injury during recovery). In addition, claudication is associated with the release of powerful vasoconstrictor agents such as endothelin-1 and with a marked reduction in endothelium-mediated vasodilation.\textsuperscript{176,177}

In addition to these systemic responses, exercise triggers acute local inflammation. In 17 patients with unilateral PAD, Neumann et al\textsuperscript{178} found that immediately after claudication, total neutrophil number, neutrophil flexibility, and the proportion of activated neutrophils were higher in the venous blood draining from the affected leg than in arterial blood. These venous-arterial differences were not observed from the circulation of the contralateral unaffected exercising leg. Furthermore, activated leukocytes release thromboxane A\textsubscript{2}, which is a vasoconstrictor and promotes platelet aggregation.\textsuperscript{179} In claudicants, P-selectin, which mediates platelet-endothelium interaction, may also contribute to platelet alterations in the microcirculation.\textsuperscript{180–182}

Consistent with the report by Neumann et al, we found that in the claudicant limb, but not in the healthy legs of a control group, maximal exercise increased the transfemoral venous-arterial difference of the neutrophil myeloperoxidase content, an index of neutrophil activation and a well-established marker of inflammation.\textsuperscript{116,117,178} Myeloperoxidase is an enzyme that, when released by activated neutrophils, exerts noxious effects on the endothelium.\textsuperscript{117} In particular, it uses nitric oxide as a physiological substrate, thereby reducing the bioavailability of nitric oxide that is fundamental for both vasodilation and modulation of adhesion molecule expression. In addition to myeloperoxidase, activated neutrophils release various toxic substances, among which is elastase. This molecule has been shown to exert harmful effects on the endothelium in vitro, and its levels increase progressively in healthy individuals to asymptomatic PAD patients to claudicants.\textsuperscript{183,184} Furthermore, in claudicants, its levels increase further with exercise.\textsuperscript{185} In patients with claudication, these inflammatory responses to exercise may have adverse interactions with both the microcirculation and skeletal muscle metabolism that could further compromise exercise performance.

**Alterations in Skeletal Muscle Structure**

Various morphological alterations have been identified in the skeletal muscle of PAD patients, including muscle apoptosis and atrophy, increased fiber type switching from oxidative type I fibers to glycolytic type II fibers, muscle fiber denervation, altered myosin heavy chain expression, and mitochondrial DNA injury.\textsuperscript{186–190} Increasing evidence indicates that inflammatory mediators play an important role in skeletal muscle wasting and fatigue. Tumor necrosis factor-\textalpha and interleukin-6, which are increased in PAD, induce skeletal muscle protein breakdown in rats and are negatively related to muscle mass and muscle strength in elderly individuals.\textsuperscript{174,191–193} Furthermore, tumor necrosis factor-\textalpha may provoke apoptosis in skeletal myocytes.\textsuperscript{194} McDermott et al\textsuperscript{195} found that in PAD, higher levels of inflammatory markers (CRP, interleukin-6, and soluble vascular cell adhesion molecule-1) were associated with a small calf area, and interleukin-6 and soluble vascular cell adhesion molecule-1 were associated with a higher percent of calf muscle fat. However, the impact of these observations on muscle functions and exercise performance in PAD has not been established.

**Inflammation and the Hemodynamic and Clinical Severity of PAD**

The pathophysiology of intermittent claudication is attributed primarily to flow-limiting stenosis or occlusions in conduit arteries and impaired vasodilation in resistance vessels that attenuate the requisite blood flow augmentation and maintenance of pulsatile flow to ischemic areas of exercising skeletal muscle. In addition, some studies indicate that the reduced walking ability of affected individuals may be influenced by other factors, many of which are linked to inflammation.\textsuperscript{18,116,118,121,172–174,178,187,188,196–199} Furthermore, inflammation may be a marker of the severity of PAD as defined by the ABI, which is a measure of the degree of limb hemodynamic compromise resulting from atherosclerotic obstruction. In the Rotterdam study, in multivariate analysis, logarithmically transformed CRP and interleukin-6 were inversely related to ABI after adjustment for age, smoking status, body mass index, and diabetes mellitus.\textsuperscript{200} In the Edinburgh Artery Study, fibrinogen was independently and negatively associated with ABI.\textsuperscript{184} Furthermore, the National Health and Nutrition Examination Survey 1999 to 2002 showed that geometric mean CRP, fibrinogen, and leukocyte count were incrementally greater with each successively lower ABI category.\textsuperscript{21} In terms of longitudinal data, Aboyans et al\textsuperscript{201} studied 403 subjects, 44.9% of whom were affected by PAD. Toe-brachial index and ABI were measured at baseline and after a mean follow-up of 4.6±2.5 years. The highest decile of decline of the pressure indexes was consid-
erated major progression, which was a $-0.30$ ABI decrease for large-vessel PAD and a $-0.27$ toe-brachial index decrease for “small-vessel” PAD progression. Of the 403 subjects enrolled in the study, 43 (10.6%) had an ABI decrease exceeding $-0.30$. Multivariate analysis showed that lipoprotein(a) and high-sensitivity CRP were independent and significant predictors of large-vessel PAD progression. Of the 290 subjects who underwent toe-brachial index measurement, 29 (10.0%) showed major progression of small-vessel PAD. In these patients, however, the cutoff of $-0.27$ toe-brachial index did not identify any inflammatory marker as a predictor. In the Edinburgh Artery Study, CRP, interleukin-6, and soluble intercellular adhesion molecule-1 levels were significantly associated with an ABI decrease at 12 years independently of baseline ABI and cardiovascular risk factors. Thus, inflammation may contribute to the primary pathophysiology of lower-extremity atherosclerosis. In contrast to these data are those of the Homocysteine and Progression of Atherosclerosis study, which reported no association between CRP and PAD progression, defined as a decrease in ABI $>0.15$ over a mean follow-up of 38.4 months.

Inflammation may also contribute to the severity of PAD symptomatic manifestations. For example, interleukin-6 was inversely correlated with maximum treadmill walking distance independently of other risk factors. Further evidence of a link between inflammation and PAD severity is the finding that levels of inflammatory markers parallel the staging of the Fontaine classification. In a small study comparing 19 patients at Fontaine stage I (ie, asymptomatic PAD) with 19 claudicants (Fontaine stage II), plasma levels of high-sensitivity CRP and interleukin-6 were significantly higher in stage II patients than in stage I patients, and the latter group did not differ from the control group.97 Plasma levels of soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 did not differentiate the 2 PAD groups. Notably, each claudicant was matched to an asymptomatic PAD patient for several factors known to affect inflammatory status. Thus, the between-group differences in the levels of inflammatory markers were probably due to PAD symptomatic severity. Cassar et al26 studied 132 claudicants, 30 patients with critical limb ischemia, ie, patients with rest pain (Fontaine stage III), and patients with trophic lesions (Fontaine stage IV) and found that high-sensitivity CRP levels in claudicants (3.4 mg/L) were intermediate between those of a control group (1.0 mg/L) and those of patients with critical limb ischemia (7.2 mg/L). Consistent with the previous 2 studies, CRP and $\alpha_1$ acid glycoprotein (another acute-phase reactant) progressively increased in PAD patients with worsening clinical Fontaine stage.

More direct evidence that inflammation is associated with lower-extremity functioning comes from a series of studies based on function tests. In 370 men and women with PAD and 231 without PAD, McDermott et al205 determined the association between 3 inflammatory markers (CRP, fibrinogen, and serum amyloid A) and the following function outcomes: 6-minute walk distance, 4-m walking velocity, and a summary performance score that combined performance in walking speed, standing balance, and time for 5 repeated chair rises. No association was observed in subjects without PAD. Conversely, in PAD patients, CRP levels, but not fibrinogen or serum amyloid A levels, were independently related to 6-minute walk distance and summary performance score. In a subsequent study of 188 PAD patients, the same group reported that physical activity levels were independently and inversely associated with fibrinogen ($P=0.014$) and log serum amyloid A ($P=0.012$).

All the above-mentioned studies were cross-sectional and do not fully address the independent contribution of inflammation over and above the arterial disease severity as contributing to functional and symptomatic disease severity. In addition, there are only a few prospective evaluations of the relationship between inflammation and PAD clinical severity progression. For example, in PAD patients, greater annual increases in CRP were associated with a greater annual decline in 6-minute walk performance over a period of 3 years.

Therefore, with only a few exceptions, cross-sectional and prospective studies show a strong relationship between elevated serum or plasma levels of various inflammatory markers (prevalently CRP and interleukin-6) and PAD claudication severity. However, cross-sectional studies and prospective studies that are observational in nature cannot establish the causality of the reported association. Therefore, it remains to be clarified whether primary systemic inflammation is responsible for more severe intermittent claudication or whether it is extensive, severe atherosclerosis of the leg arteries that contributes to the increased inflammatory status. Obviously, the 2 hypotheses are not mutually exclusive. In any case, given the negative prognostic impact of inflammation, the above findings suggest that an increased inflammatory status could explain the increasingly poor prognosis as PAD becomes more severe.8,135–147,208,209 However, further studies are needed to verify this hypothesis.

Conclusions

Appreciation of the role of inflammation in PAD has burgeoned over the last decade. From the literature available, there is no doubt that an increased inflammatory status is associated with the development and subsequent worsening of atherosclerosis in the arteries of the lower limbs and is involved in the extent and severity of vascular disease in other arterial territories. Particularly intriguing with respect to vascular comorbidity is the hypothesis that a pronounced intravascular inflammatory process of the affected limb, caused by the presence of multiple “active” plaques, could be associated with more severe coronary and carotid atherosclerosis by a distinct pathophysiological mechanism. Should the mechanistic action of local endovascular inflammation in PAD be confirmed, new therapeutic approaches could prevent the development or worsening of atherosclerosis in other vascular districts.

In addition to its pathophysiological implications, the severity of inflammation also predicts future ischemic events, such as myocardial infarction and stroke. Remarkably, some of the inflammatory molecules, beyond their individual predictive value, improve the risk stratification provided by ABI, which is to date the most powerful prognostic indicator in established PAD. This may be not only relevant from a
therapeutic point of view but also especially useful to address diagnostic strategy. Actually, many PAD patients are affected by relevant coronary atherosclerosis, although often in asymptomatic form. In these cases, the presence of elevated levels of inflammatory markers could be useful to identify candidates for further diagnostic evaluations and more intensive risk modification.

However, the inflammatory mechanisms surrounding PAD are only just emerging. The most pertinent question is the origin of the inflammatory response and its role in the course of PAD symptomatic manifestations and comorbidity. It is important to distinguish between systemic and local inflammation and to evaluate to what extent local inflammation is involved. Additional research is critical for progress in this area and the development of specific therapies to improve the quality of life and outcome of PAD patients.

Acknowledgments

Dr Brevetti tragically died in December 2009, and thus, this is the last major work of his outstanding academic career. We are grateful to Jean Ann Gilder for language editing and for her support to Dr Brevetti during the preparation of the manuscript.

Disclosures

Dr Hiatt reports previously receiving grant support to his research team. Dr Brevetti during the preparation of the manuscript.

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**KEY WORDS:** atherosclerosis • claudication • inflammation • microcirculation • peripheral vascular disease • risk factors • vessels
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Circulation. 2010;122:1862-1875
doi: 10.1161/CIRCULATIONAHA.109.918417
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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