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Novel markers of peripheral arterial disease

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Abstract

Peripheral arterial disease (PAD), a relatively common manifestation of atherosclerotic vascular disease, is associated with significant morbidity and mortality. Although conventional risk factors contribute to the onset and progression of PAD, the role of 'novel' biomarkers in pathways of inflammation, thrombosis, lipoprotein metabolism, and oxidative stress in determining susceptibility to PAD is being increasingly recognized. Validation of novel risk factors for PAD may allow earlier detection, an improved understanding of disease etiology and progression, and the development of new therapies. In this review, we discuss available evidence for associations between novel circulating markers and several aspects of PAD including disease susceptibility, progression, functional limitation, and adverse outcomes.

Keywords

inflammation; peripheral arterial disease; risk factors

Introduction

Atherosclerotic vascular disease affects large- and medium-sized arteries of most circulatory beds and is the leading cause of death and disability in developed countries. Lower-extremity atherosclerotic peripheral arterial disease (PAD) is a significant public health problem in the USA, with an estimated 8–10 million affected individuals.[1] Although conventional risk factors are known to contribute to the development of PAD, the role of 'novel' bio-markers in pathways of inflammation, thrombosis, lipoprotein metabolism, and oxidative stress in determining susceptibility to PAD is not fully defined. Validation of novel risk markers for PAD may allow earlier detection, an improved understanding of disease etiology and progression, and the development of new therapies.

Since PAD may have varying clinical presentations, a valuable tool for investigating novel markers for this disease is the ankle–brachial index (ABI), an objective, reproducible, non-invasive measure that correlates with PAD severity.[2] An ABI ≤ 0.90 is 95% sensitive and 90% specific for the presence of a $\geq 50\%$ narrowing of a lower extremity artery and is used in the clinical setting to establish a diagnosis of PAD.[3] The change in ABI over time provides a measure of PAD progression and ABI also provides prognostic information; patients with severe PAD (ABI ≤ 0.40) have a significantly decreased survival, with only 24% of patients

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alive at 12 years.[4] However, ABI is not strongly correlated with functional capacity in patients with PAD.[5–7]

In recent years, several 'novel' circulating markers, including C-reactive protein (CRP), fibrinogen, lipoprotein(a) (Lp(a)), and homocysteine, have been examined as potential risk factors for atherothrombotic vascular disease. This review will focus on the association of these and additional markers with PAD, including markers of inflammation, thrombosis, lipoprotein metabolism, and oxidative stress (Table 1). Where data are available, we discuss these markers not only as predictors of onset of PAD, but also progression, functional capacity, and adverse outcomes in PAD patients.

Inflammation

A substantial body of evidence has accumulated to support a key role for inflammation in the development and progression of atherosclerosis. Several studies have investigated the association of various markers of vascular inflammation including acute phase reactants (CRP), cytokines (interleukin-6 (IL-6)), cellular adhesion molecules (CAMs), white blood cell (WBC) count, and beta2-microglobulin, with manifestations of atherosclerotic vascular disease.

C-reactive protein (CRP)

CRP is an acute phase reactant and plasma levels increase in response to inflammatory processes. It is a pentameric protein primarily produced in the liver in response to IL-6 and other inflammatory cytokines.[8] Recent data suggest that CRP is also synthesized in the smooth muscle cells of atherosclerotic plaques.[9] Initially thought to simply be a marker of systemic inflammation, CRP may be mechanistically involved in atherosclerotic plaque development and instability. Putative mechanisms of vascular damage due to CRP include down-regulation of endothelial nitric oxide synthase, increased endothelial expression of cell adhesion molecules (CAMs), activation of the complement system, upregulation of low-density lipoprotein (LDL) phagocytosis by macrophages, and vascular smooth muscle cell migration, proliferation, and neo-intimal formation.[10–17]

Not only has CRP been shown to be an independent predictor of myocardial infarction and stroke in asymptomatic individuals,[18,19] it was also predictive of onset of symptomatic PAD in a cohort of 14,916 mostly white men (ages 40–84 years) followed for a mean of 9 years[20, 21] and in a prospective observational study of 27,935 women aged ≥ 45 years followed for a mean of 12 years.[22] Several studies also investigated the relationship between the ABI and CRP. A higher CRP level was associated with a lower ABI in several studies, although the relationship was attenuated after adjustment for conventional risk factors and physical activity.[23–30] In the National Health and Nutrition Examination Survey (NHANES), CRP was not significantly associated with PAD (ABI ≤ 0.9) after adjustment for measures of insulin resistance;[31] and in the Edinburgh Artery Study,[32] CRP was not significantly associated with PAD after adjustment for plasma levels of IL-6. Further, among symptomatic PAD patients, CRP levels were not found to be significantly different in varying grades of claudication severity, implying that CRP may not be related to the severity of obstructive PAD.[33] Given that both elevated CRP as well as a low ABI have been associated with increased risk of cardiovascular events [34,35] and the two do not appear to be strongly correlated, and may predict risk through independent pathways, measurement of both may provide incremental information. CRP levels may be a marker of plaque instability and vascular inflammation that leads to disease progression and adverse cardiovascular outcomes, whereas ABI is a marker of atherosclerotic burden.

CRP has also been studied in PAD patients as a marker of disease progression, functional activity, and adverse cardiovascular events such as myocardial infarction and stroke. Plasma

levels of CRP predicted progression of PAD (as determined by a decrease in ABI over time) in two large population-based longitudinal cohort studies: the Rotterdam Study (n = 7987, mostly white men and women in The Netherlands; age ≥ 55; follow-up 6.5 years)[36] and the Edinburgh Artery Study (n = 1592, mostly white men and women in the UK; ages 55–74; follow-up 5–12 years),[32] as well as two smaller studies, over a period of 3–12 years.[37,38] Inflammation also appears to be associated with a decline in function and physical activity often seen in patients with PAD and resulting in a significant impact on quality of life. Higher levels of CRP were correlated with impaired functional capacity, decreased physical activity, and future functional decline in several cohort studies.[39–43]

CRP is predictive of adverse cardiovascular events in PAD patients,[33,44] including those undergoing revascularization.[33] An elevated CRP was also predictive of all-cause and cardiovascular mortality at 1 year but not at 2–3 years in a prospective cohort study of 377 patients with PAD.[45] These data suggest that inflammation may be related to plaque instability in patients with PAD. In a prospective study of 110 patients referred to a non-invasive vascular laboratory, CRP provided prognostic information in addition to ABI.[46] A high CRP (> 3.0 mg/l) and a low ABI (ABI < 0.9) were associated with an odds ratio of 2.6 and 4.0, respectively, for adverse cardiovascular events (myocardial infarction, stroke, coronary or lower extremity revascularization, or death); when both were present, the odds ratio increased to nearly 6. Combined use of a marker of inflammation and ABI may therefore have a greater predictive value for cardiovascular events than either measure alone.

CRP may be predictive of the risk of developing symptomatic PAD independent of conventional risk factors, but further studies are needed to confirm its clinical utility in this regard. Perhaps of greater clinical relevance is the fact that circulating CRP levels appear to be predictive of cardiovascular events and mortality in patients with known PAD and may also be associated with disease progression and functional decline. Whether lowering of plasma CRP levels will benefit patients with PAD is not known. Several interventions have been shown to reduce CRP levels including diet,[47] moderate alcohol consumption,[48] statins,[49,50] fibrates,[51] and fish oil.[52] A small molecule that inhibits CRP-related complement activation has been synthesized and was shown to reduce the size of myocardial infarction in rats.[8] Prospective randomized controlled trials are needed to investigate whether lowering CRP levels reduces disease progression, mortality, or functional decline in PAD.

Interleukin-6 (IL-6)

IL-6 is an inflammatory cytokine produced by hepatocytes, lymphocytes, and endothelial cells.[53] Whether IL-6 is associated with incident PAD, PAD severity, or cardiovascular events in PAD patients is not known. However, in the Edinburgh Artery Study, IL-6 was the strongest predictor of PAD progression over a 12-year period, and was the only biomarker to remain independently associated with disease progression after all ‘novel’ bio-markers and conventional risk factors were included in the analysis.[32] IL-6 is the stimulus for CRP production in the liver, and it is more proximal in the inflammatory cascade. However, it is less abundant in the plasma and the assays are not as robust, diminishing its potential for clinical use. The findings of several studies suggest that IL-6 levels may be regulated by the level of physical activity. Although IL-6 levels are significantly increased after exercise in PAD patients,[54] a higher IL-6 level has been associated with lower functional capacity in such patients.[55]

Cellular adhesion molecules

Cellular adhesion molecules (CAMs), expressed on the vascular endothelium and circulating leukocytes, mediate recruitment of leukocytes to the vascular wall and into sub-endothelial spaces and are implicated in atherogenesis.[56] Brevetti et al.[56] have summarized the evidence

for CAMs as markers for the presence, severity, extent of PAD, and the risk of adverse cardiovascular events. In the Physicians' Health Study, soluble intercellular adhesion molecule-1 (sICAM-1) was associated with an increased risk of developing symptomatic PAD in men,[57] and similar findings were reported in the Women's Health Study.[22] In the Edinburgh Artery Study, sICAM-1 predicted PAD progression over a 12-year period.[32] Khaleghi et al. reported that sICAM-1 and soluble vascular cell adhesion molecule-1 (sVCAM-1) were associated with the presence of PAD in African Americans, but not in non-Hispanic whites.[58] There are no therapies that specifically lower CAMs although statins have been shown to decrease plasma CAM levels.[59] Succinobucol, a potent antioxidant that may inhibit CAMs, did not reduce adverse cardiovascular events (death, cardiac arrest, MI, stroke, unstable angina, or coronary revascularization) after myocardial infarction.[60] Clinical trials of this novel agent and other interventions that reduce CAM levels have yet to be performed in PAD patients.

WBC count

In the NHANES (1999–2002), a significant cross-sectional association of inflammatory markers (WBC count, fibrinogen and CRP level) with the presence of PAD (ABI < 0.9) was noted.[61] A WBC count in the top quartile ($> 7.3 \times 10^3/mm^3$) was associated with an odds ratio of 1.67 for PAD compared to the bottom quartile ($\leq 4.9 \times 10^3/mm^3$). A subsequent analysis of the NHANES database found that monocytes were the only WBC subtype significantly and independently associated with PAD.[62] Grau et al.[63] in a study of more than 18,000 patients with known atherosclerotic vascular disease (history of stroke, myocardial infarction, or symptomatic PAD) found that WBC count $> 8.2 \times 10^3/mm^3$ (top quartile) was associated with a higher risk of vascular death (RR = 1.51) when compared with the WBC count $\leq 5.85 \times 10^3/mm^3$ (bottom quartile). The association of WBC count with all-cause mortality was not reported. Haumer et al.[64] followed 398 patients with symptomatic PAD (requiring revascularization or salvage for critical limb ischemia) for a median of 20 months. Compared to patients with a neutrophil count < 4.4 ($\times 10^3/mm^3$) (bottom tertile), those with a neutrophil count > 5.8 ($\times 10^3/mm^3$) (highest tertile) were at a higher risk of major adverse cardiovascular events (myocardial infarction, need of percutaneous intervention, coronary bypass, stroke, carotid revascularization) (HR = 1.8) and death (HR = 3.4) and composite of myocardial infarction, stroke and death (HR = 2.2).

Although the WBC count could simply be a marker of inflammation in the setting of atherosclerotic vascular disease, it is possible that WBC may have a pathogenic role by activating various cascades, favoring plaque progression and rupture leading to acute coronary (or other ischemic) syndromes.[65,66] The possible mechanisms by which an elevated WBC count may be related to adverse outcomes have been reviewed by Madjid et al.[67] and Coller[66] and include increased inflammation, endothelial damage, procoagulant effects and microvascular damage. Indeed, Coller has proposed a clinical trial using agents that reduce the WBC count, such as hydroxyurea, in patients with atherosclerotic vascular disease and increased risk of death.[66]

Beta2-microglobulin

Beta2-microglobulin is a component of the major histocompatibility complex class I molecule. Wilson et al.[68] performed comparative proteomic profiling of PAD patients and controls and found that serum levels of beta2-microglobulin were higher in PAD than in non-PAD patients. This finding was validated by the investigators in 237 subjects referred for coronary angiography who were also screened for PAD. Plasma beta2-microglobulin levels were higher in PAD patients than in non-PAD patients with coronary artery disease and beta2-microglobulin levels were independently associated with ABI.[68] Further studies are needed to determine the clinical utility of this biomarker.

Other inflammatory markers

The association between PAD and several other inflammatory markers has been examined in a limited number of studies. In the Atherosclerotic Risk in Communities (ARIC) study, monocyte chemoattractant protein-1 (MCP-1) was associated with an ABI ≤ 0.90.[69] MCP-155 and soluble CD40L[55,70] were associated with the angiographic severity of PAD. Myeloperoxidase (MPO) was associated with adverse cardiovascular events in a cohort of 156 patients with PAD,[71] and with an ABI ≤ 0.90 in a bi-ethnic cohort of mostly hypertensive adults.[72] Serum osteoprotegerin was also associated with an ABI ≤ 0.90 in the latter cohort.[73] Circulating neopterin[74] and tumor necrosis factor alpha (TNF-α) levels[74] were found to be associated with 1-year mortality in patients with PAD.

Thrombosis

Thrombosis serves an important biologic function to reduce intravascular blood loss through the activation of the clotting cascade, which leads to fibrin and platelet deposition at the site of vascular injury. This cascade has been implicated in the formation and progression of atherosclerotic plaque as well as in the propensity towards acute cardiovascular events. Several markers of thrombosis including fibrinogen and D-dimer have been studied to characterize the relationship between thrombosis and PAD, whereas the association between PAD other thrombotic markers including von Willebrand factor (vWF), tissue plasminogen activator (tPA), and plasminogen activator inhibitor (PAI-1) is less well established.

Fibrinogen

Fibrinogen – a glycoprotein synthesized in the liver –is a key component of thrombus formation, a known acute phase reactant, and also a major determinant of plasma viscosity. Fibrin, the cleaved product of fibrinogen, plays an essential role in platelet adhesion and aggregation in clot formation. Fibrinogen levels are in part genetically determined,[75] and are also increased with age, obesity, diabetes, menopause,[76] and smoking.[77] A number of prospective cohort studies have shown plasma fibrinogen levels to be an independent predictor of future onset of PAD.[21,78,79] Plasma fibrinogen was predictive of the development of claudication in the Framingham Heart Study and is included in the Framingham equation to assess the risk of PAD.[80]

Higher fibrinogen levels have also been independently associated with a lower ABI in most[24,81–85] but not all[29] cross-sectional cohort studies. A higher plasma fibrinogen level was associated with a lower ABI in both African Americans and non-Hispanic whites belonging to sibships ascertained on the basis of hypertension.[27,86] In the Edinburgh Artery Study, plasma fibrinogen and a history of smoking had a synergistic effect in reducing ABI.[87] In the same study, the fibrinogen level was predictive of progression of PAD over 12 years, although this association was attenuated after adjustment for markers of inflammation including CRP and IL-6.[88] In contrast to CRP, fibrinogen has not been independently associated with physical activity levels or other measures of lower extremity functioning in patients with PAD.[39–42] However, fibrinogen levels are independently predictive of cardiovascular mortality in PAD patients.[89]

Fibrinogen levels are reduced by smoking cessation, exercise, and alcohol.[90] Among drugs, estrogens lower fibrinogen levels in contrast to their effects on raising CRP levels.[91] Fibrates have significant fibrinogen-lowering effects,[92] but because they concomitantly modify plasma lipids, these drugs are not ideal for trials to determine the specific effects of fibrinogen lowering. In a randomized controlled trial of bezafibrate in men with PAD, concomitant lowering of fibrinogen and triglyceride levels led to a significant reduction in non-fatal myocardial infarction, although there was no reduction in fatal myocardial infarction or stroke.[93]

D-dimer

The relationship between D-dimer, a product of fibrin degradation by plasmin, and PAD is controversial. An elevated D-dimer has been associated with a lower ABI in several cross-sectional studies.[24,29,94] In the Edinburgh Artery Study, D-dimer was not independently associated with PAD progression after adjustment for CRP and IL-6 levels.[88] However, participants with the highest D-dimer and IL-6 levels had the greatest decline in ABI over a 12-year period.[88] McDermott et al. have demonstrated an elevated D-dimer to be associated with walking impairment,[41] functional capacity,[39] physical activity levels,[40] and functional decline over time.[42] Whether D-dimer is involved in the pathophysiology of functional impairment in PAD patients or is simply a marker of the same is not established.[38] In a study of 384 subjects designed to examine the effect of risk factors on PAD progression, D-dimer was associated with an increased risk of myocardial infarction, but not with PAD progression. In another prospective study of 377 men and women with PAD, D-dimer was associated with all-cause mortality during the first year of follow-up but not 2–3 years after biomarker measurement, suggesting that this biomarker may be a better predictor of short-term rather than long-term outcomes.[45]

Other thrombotic markers

Higher levels of vWF were associated with a lower ABI in a cross-sectional analysis of the ARIC study [95] but not with the development of PAD in the Edinburgh Artery Study[96] or the ARIC study.[79] Neither tPA or plasminogen activator inhibitor-1 (PAI-1) were associated with ABI in the ARIC study[95] and in another cross-sectional study.[29] In the Edinburgh Artery Study, tPA was only weakly associated with the development of PAD. The prognostic value of PAI-1 in the setting of PAD is not known. These two markers were also not associated with physical activity levels in PAD patients.[40]

Lipoprotein markers

LDL cholesterol is an established risk factor for atherosclerotic vascular disease and a major therapeutic goal in PAD patients is the reduction of LDL cholesterol levels. New evidence suggests that several novel lipoprotein markers may also be associated with the development and progression of atherosclerotic vascular disease including PAD.

Lipoprotein-associated phospholipase A2 (Lp-PLA_2)

Lp-PLA_2 is a novel marker of increased risk of coronary heart disease (CHD) and stroke.[97] Whether Lp-PLA_2 is associated with future risk of PAD is not known. In a study of 1820 community-based subjects based in Rotterdam, Lp-PLA_2 was associated with an ABI < 0.90; however, the association was no longer present after adjustment for total and high-density lipoprotein (HDL) cholesterol.[98] In a prospectively followed cohort of PAD patients, Lp-PLA_2 was not associated with adverse cardiovascular events.[99] The available data suggest that Lp-PLA_2 is not associated with PAD or cardiovascular mortality in PAD patients.

Lipoprotein(a) (Lp(a))

Lp(a) is a circulating lipoprotein that resembles LDL in that both molecules have apo B-100 as the surface apolipoprotein. In addition, Lp(a) has a unique glycoprotein, apo(a), bound to apo B-100 by a disulfide bond; apo(a) resembles plasminogen and competitively binds to plasminogen receptor sites.[100] Several mechanisms have been proposed for the atherogenic effects of Lp(a), including inhibition of plasminogen activity related to the structural homology of apo(a) to plasminogen,[100] induction of PAI-1, inhibiting release of tPA,[101,102] and vascular smooth muscle cell proliferation.[103]

Studies investigating the association between Lp (a) levels and PAD have yielded conflicting results. The Physicians' Health Study[21] did not find Lp(a) levels to be predictive of symptomatic PAD. However, several cross-sectional, case–control studies have found an association between elevated Lp(a) levels and PAD in various populations including those with premature PAD (men aged < 45),[104,105] those with known PAD,[106,107] and those referred for lower extremity revascularization.[108] Other cross-sectional cohort studies have found significant associations between Lp(a) levels and PAD (ABI < 0.90) in the elderly (n = 369),[109] and in type II diabetics (n = 557).[110] Additionally, a prospective longitudinal observational study found Lp(a) to be an independent predictor of progression of PAD (as determined by a decrease in ABI over 4.6 ± 2.5 years).[37]

Plasma levels of Lp(a) largely depend on the size of the apo(a) isoform present, and are inversely related to the number of apo(a) kringle IV repeats.[111] Elevated Lp(a) levels may be more atherogenic in the presence of small apo(a) size (defined as < 22 kringle 4 (K4) repeats) versus larger apo(a) isoforms.[112] In African Americans, high Lp(a) levels are less likely to be associated with the presence of small apo(a) isoforms than in Caucasians.[113] This observation may explain why elevated Lp(a) levels have not been consistently associated with increased cardiovascular risk in African Americans.[114] In one study,[115] elevated levels of Lp(a) with small apo(a) isoforms were associated with the angiographic extent of coronary artery disease in both African American and Caucasian men. In a bi-ethnic cohort of mostly hypertensive individuals, higher Lp(a) levels were associated with PAD in African Americans but not non-Hispanic whites.[27]

Pharmacologic options for lowering elevated Lp (a) levels are limited. Statins do not appear to have a significant effect on Lp(a) levels.[116] However, lowering of LDL cholesterol levels may diminish the risk of adverse cardiovascular events due to elevated Lp(a).[117] Estrogens decrease plasma Lp(a) concentration,[118] and a post hoc analysis from the Women's Health Study revealed that estrogens had a favorable impact on cardiovascular events in the women who had elevated Lp(a) levels.[119] Niacin has been shown to have Lp(a)-lowering effects, alone[120] and in conjunction with neomycin[121] and statins.[122] No clinical trials have been performed to confirm whether a decrease in Lp(a) levels leads to improved cardiovascular outcomes, in part because drugs that specifically and significantly lower Lp(a) are not yet available.

Other markers

Homocysteine

Homocysteine is an amino acid intermediate produced during the metabolism of methionine.[123] Its role in atherogenesis was proposed subsequent to the observation that children and young adults with cystathione beta-synthase deficiency and markedly elevated homocysteine levels developed premature atherosclerosis. Homocysteine may promote atherosclerosis through several mechanisms, including increased oxidant stress and adverse effects on endothelial function.[123] Increased homocysteine levels may result from genetic polymorphisms in enzymes related to its metabolism, as well as aging, menopause, hypothyroidism, low B vitamin and folic acid levels, and chronic kidney disease.[124–128]

Several studies found homocysteine levels to be associated with PAD, the Physicians' Health Study being a notable exception.[21] In a cross-sectional population-based study, higher homocysteine levels were associated with a reduced ABI (<0.90).[129] Elevated homocysteine levels were also associated with a lower ABI in a multiethnic cohort of predominantly hypertensive adults,[27] with symptomatic PAD in a referral population,[108] and with angiographic severity of PAD in type II diabetic patients.[130] A meta-analysis found elevated homocysteine levels (> 15 μmol) to be related to atherosclerotic disease in several vascular

beds, but most strongly to PAD.[131] Additionally, elevated homocysteine levels predict all-cause and cardiovascular death in patients with PAD.[132,133] Of note, in an analysis of 4447 NHANES participants,[134] the association between PAD and plasma homocysteine was attenuated after adjustment for plasma levels of the heavy metals lead and cadmium, suggesting that homocysteine may be a marker of an as yet unknown atherogenic factor.

Vitamins B6, B12, and folic acid reduce homocysteine levels; however, the Heart Outcomes Prevention Evaluation (HOPE2) study found that B vitamins do not reduce the risk of major cardiovascular events (excluding stroke) in patients with vascular disease.[135] Another recent trial of women at high risk of developing cardiovascular disease demonstrated that lowering homocysteine with B vitamins and folic acid did not lower cardiovascular mortality.[136] The results of these trials indicate that treating homocysteine levels with B vitamins and folate in patients with known vascular disease is not currently warranted. However, the HOPE2 findings do not diminish the fact that homocysteine is a marker for increased cardiovascular risk, especially PAD. The role of homocysteine in PAD appears to be complex, and further studies are needed to better understand the relationship of homocysteine and atherothrombosis, and whether pharmacologic agents (other than folate) that reduce homocysteine levels can alter cardiovascular outcomes.

Asymmetric dimethylarginine (ADMA)

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase, the enzyme that produces nitric oxide, a vasodilator with anti-inflammatory and anti-thrombotic effects. Plasma ADMA levels were elevated in patients hospitalized with symptomatic PAD[137] and predictive of adverse cardiovascular events (myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, carotid revascularization, and death) in such patients.[138] Infusion of L-arginine (the substrate for nitric oxide production) improved walking distances and flow-mediated dilation in a study of patients with PAD.[139] However, a recent randomized controlled trial found no evidence of benefit in patients with PAD.[140]

Oxidative stress

Oxidative damage to lipids and proteins is implicated in the development of atherosclerotic vascular disease.[141] To date, the data pertaining to any possible association between PAD and other markers of oxidative stress are sparse. In small, case–control studies, plasma levels of glutathione peroxidase-1 activity,[142] 8-iso-prostaglandin F2α,[143] and vitamin C (L-ascorbic acid)[29] have been associated with PAD. This is clearly an area that requires further investigation.

Matrix remodeling

Matrix metalloproteinases (MMP) and their inhibitors are markers of extracellular matrix turnover and have been associated with PAD in two case–control studies. The first study included 36 patients with intermittent claudication, 43 with critical limb ischemia, and 42 controls.[144] The second study included 51 type II diabetics with PAD, 42 type II diabetics without PAD, and 23 controls.[145] Pregnancy-associated plasma phosphatase (PAPP-A) is a zinc-binding metalloproteinase that was associated with PAD in a case–control study of 433 PAD patients and 433 controls.[146]

Angiogenesis

Vascular endothelial growth factor (VEGF) was associated with PAD in two case–control studies; the first included 70 patients with PAD and 70 controls,[143] and the second study included 234 patients with an ABI $\leq$ 0.8 and 50 controls.[147] Levels of the VEGF receptor,

soluble Flt-1, were associated with PAD in the first study,[143] but not in the second one.[147] Plasma angiopoietin2 was increased in both intermittent claudication and critical limb ischemia compared with controls ($n = 23$ for intermittent claudication, $n = 23$ for critical limb ischemia, $n = 23$ for healthy controls).[148] A receptor tyrosine kinase specifically expressed in developing vascular endothelial cells, tie-2, plays an important role in angiogenesis. Soluble tie-2 levels were increased in patients with critical limb ischemia compared with controls ($n = 46$ patients with PAD, $n = 23$ controls).[148]

Conclusion

Considerable evidence supports the association of novel circulating markers with several aspects of PAD including: (1) the risk of developing symptomatic or asymptomatic PAD; (2) progression of PAD; (3) functional impairment; and (4) adverse cardiovascular events and mortality. However, significant gaps in knowledge remain. Given the complexity of atherosclerotic vascular disease, a single marker is unlikely to yield significant predictive or prognostic information and a multimarker approach is more likely to be useful. It is clear from this review that there are robust data to support an association of inflammation and thrombosis with PAD; whereas the data regarding the association of novel markers of lipoprotein metabolism, oxidative stress, and angiogenesis are still evolving.

New circulating markers could provide incremental information about the risk of developing PAD and add prognostic information beyond what is possible with the measurement of the ABI, particularly in the prediction of cardiovascular events and mortality.[27,149] Such knowledge could also lead to a better understanding of disease pathways with significant diagnostic and therapeutic implications. Further work is needed to develop clinically useful markers of PAD including novel assay platforms and statistical methodology, targeted approaches using candidate protein in etiologic pathways of disease, as well as an agnostic approach using discovery proteomics.

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Table 1

Novel circulating markers that have been implicated in PAD

Inflammatory markers
C-reactive protein
Interleukin-6
Cellular adhesion molecules
White blood cell count
Beta2-microglobulin
Monocyte chemoattractant protein-1
Soluble CD40 ligand
Myeloperoxidase
Neopterin
Osteoprotegerin
Tumor necrosis factor-alpha
Thrombotic markers
Fibrinogen
D-dimer
von Willebrand factor
Tissue plasminogen activator
Plasminogen activator inhibitor
Lipoprotein metabolism
Lipoprotein-associated phospholipase A2
Lipoprotein(a)
Oxidative stress markers
Glutathione peroxidase 1 activity
8-iso-prostaglandin F2a
Vitamin C (L-ascorbic acid)
Matrix remodeling
Matrix metalloproteinases and their inhibitors
Pregnancy-associated plasma phosphatase
Angiogenesis
Vascular endothelial growth factor
Flt-1
Angiopoietin
Tie-1
Other markers
Homocysteine
Asymmetric dimethylarginine