The role of novel atherosclerosis markers in peripheral artery disease: is there a gender difference?

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Abstract

Peripheral arterial disease (PAD) represents a major public health problem due to its high and increasing prevalence, worldwide distribution, and significant morbidity and mortality rate. Female gender is a risk factor for PAD globally and especially in low-income countries. In this review, we summarise the present knowledge regarding the role of novel atherosclerosis markers in the development of PAD in women. We discuss inflammatory markers, cytokines, cellular adhesion molecules, markers of oxidative stress and other circulating markers, and their role in the prediction of presence, severity and complications of PAD, with particular emphasis on gender. Although many PAD biomarkers are indicative of PAD in both males and females, some are strongly correlated with the disease in females. These gender differences could be useful for the early identification and management of PAD in women.

Keywords: peripheral arterial disease, biomarkers, risk factors, gender

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Peripheral arterial disease (PAD) represents a major public health problem due to its high and increasing prevalence, worldwide distribution and significant morbidity and mortality rates.[1] The prevalence of PAD increases with age, especially in individuals over 75 years of age, in males, and subjects of African-American ethnicity.[2]

Figures regarding prevalence of the disease and gender distribution vary from one study to another, depending on the criteria used to diagnose PAD and geographical variations. However, global data on trends in PAD prevalence between 2000 and 2010, published by Fowkes and collaborators, show that in high-income countries, PAD prevalence is reported to be higher in men than in women, whereas in low- and middle-income countries, rates are slightly higher in women.[1] This is coupled with the fact that in developing countries the disease generally tends to affect younger age groups.[1]

Female gender is a risk factor for PAD globally, especially after the age of 65 years, with apparently higher rates in low- and middle-income countries, whereas in high-income countries, the male gender tends to be an independent risk factor for PAD, as data from the same analysis show.[1] This difference, although unlikely to stem from an excess of conventional atherosclerotic risk factors in females, may be related to other unidentified factors or even a diagnostic bias due to smaller body mass index, atypical symptoms or longer life expectancy in women.

Geo-economical differences may stem from lifestyle differences between developing countries and the industrialised world, with women in the former being more exposed to smoking and uncontrolled diabetes at a younger age. We also have to take into consideration that major differences in healthcare expenditure, and healthcare access between high- and low/middle-income countries, coupled with atypical symptoms and particular anthropometric characteristics, lead to delayed diagnosis and ill-treatment of this disease in women from less-developed countries. Unfortunately, all these factors contribute to female patients worldwide referring to the physician in more advanced stages of the disease, often presenting with critical limb ischaemia.[3]

In terms of ethno-racial distribution, several studies have shown that the highest prevalence of PAD of all ethnic groups is in African-American individuals, even after adjusting for other cardiovascular risk factors.[2,4,5] Ethnic differences are therefore unlikely to be caused by only lifestyle differences between individuals.[6]

During the first year after diagnosis, patients with intermittent claudication have a mortality rate ranging from 20 to 25%, with a five-year survival rate of less than 30%.[7,8] This is the reason why understanding and identifying the risk factors for the development of this disease are of utmost importance. Although atherosclerotic disease does not become clinically apparent until adult life, studies have shown that the onset of the atherosclerotic process is in childhood,[9] even in prenatal life.[10,11]

The two main risk factors for the development of PAD in both genders are diabetes mellitus and smoking.[12] As is the case for other cardiovascular conditions, female subjects have been under-represented in PAD clinical trials. Despite this, it was shown that women who developed PAD were older than their male counterparts and were more frequently obese and dyslipidaemic.[13-15] Other research has demonstrated the involvement of endothelial dysfunction in the pathogenesis of PAD in women. Gardner and co-workers have shown that during physical exercise, peripheral microcirculation is more deficient and the arterial elasticity indices are much lower in females with PAD compared to male subjects.[16]

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In this review, we summarise the present knowledge regarding the involvement of novel markers of atherosclerosis in the development of PAD in women.

Inflammatory markers

Although not a novel marker, fibrinogen, an important acute-phase protein, is responsible for increasing blood viscosity, with secondary prothrombotic effects.[6] Regardless of the presence of PAD symptoms, various studies have demonstrated the presence of elevated fibrinogen levels in subjects with marked peripheral atherosclerosis.[17,18] The association between high levels of fibrinogen and PAD is stronger in men than in women, while for the latter it seems to be positively correlated with smoking.[19]

The Multi-Ethnic Study of Atherosclerosis (MESA), which included individuals without a known history of cardiovascular disease, demonstrated that women of all ethnic groups had higher median levels of C-reactive protein (CRP) than men.[20] Numerous clinical trials have shown that there is a robust and independent association between CRP and the presence of PAD, regardless of gender.[17,21-23]

In a recent study, Gardner *et al.* found a direct relationship between CRP levels and the progression of PAD,[23] while Pradhan's study demonstrated a strong association between CRP, soluble intracellular adhesion molecule-1 (sICAM-1), high-density lipoprotein cholesterol (HDL-C) levels, triglyceride/HDL-C ratio and symptomatic PAD in women.[22] Female subjects with high levels of CRP enrolled in the Women's Health Study had a significantly greater risk of developing PAD over time.[17]

CRP is also a marker associated with an increased risk of developing PAD-related complications, as shown by the European Prospective Investigation into Cancer and Nutrition (EPIC): Norfolk cohort.[24] High serum levels of CRP before endovascular therapy in haemodialysis-requiring PAD patients were independently associated with increased risk of re-intervention, amputation and even overall mortality.[25]

Similar to data obtained in adults, CRP is probably the most studied inflammatory biomarker in children. Jarvisalo *et al.* found that healthy young children with higher CRP levels had higher carotid intima–media thickness (CIMT) and lower brachial artery flow-mediated dilatation.[26] The Pathobiological Determinants of Atherosclerosis in Youth Study (PDAY), which included subjects aged 15 to 34 years, demonstrated a direct relationship between CRP levels and abdominal aorta or right coronary artery atherosclerotic lesions.[27] As in older adults, CRP levels were higher in young women.[27]

Other studies, such as the Cardiovascular Risk in Young Finns study, [28] and Giannini and colleagues' work,[29] did not find an association between childhood CRP levels and adult CIMT. These findings are in line with current knowledge that high levels of inflammatory markers are independent predictors of adverse cardiovascular outcomes, and this seems to be true irrespective of gender or age.

Homocysteine

Thirty per cent of young patients with PAD have increased blood levels of homocysteine compared to 1% in the general population, as it may be a stronger risk factor for PAD than for coronary artery disease.[8] In the MESA study, homocysteine along with high levels of interleukin-6, fibrinogen and D-dimers, were significantly correlated with the presence of PAD, even after adjustment for traditional cardiovascular risk factors.[5] A meta-analysis published in 2009, comprising 14 studies, found that homocysteine levels were elevated in PAD patients compared to healthy controls.[30] Even among the PAD group, blood levels increased with age, being higher in elderly subjects.[31]

Along with other traditional atherosclerosis risk factors such as smoking, diabetes, arterial hypertension and dyslipidaemia, homocysteine is thought to be an important predisposing factor for the development of PAD in women.[32] Several older studies showed an association between high homocysteine levels and PAD in women.[33-35]

On the other hand, more recent studies give different results. Elevated levels of homocysteine had been found only in male subjects with PAD in a Japanese-Brazilian population.[36] Pradhan *et al.* found no correlation between homocysteine levels and the presence of PAD in female subjects.[22] Using data from two large cohort studies (72 348 female participants from the Nurses' Health Study and 44 504 males from the Health Professionals Follow-Up Study), of whom only subjects with clinically manifested PAD were selected, Bertoia *et al.* showed that homocysteine levels were positively associated with the risk of developing PAD only in men, not in women.[37]

In children, elevated plasma homocysteine levels correlated significantly with increased CIMT and decreased flow-mediated dilatation, but only in young girls,[38] as opposed to findings of recent adult studies. Another study enrolling adolescents with multiple risk factors for atherosclerosis proved that individuals with CIMT in the upper quartile had significantly higher mean plasma homocysteine levels than those of subjects in the lower quartile. Unfortunately, gender differences were not analysed.[39]

Lipoprotein (a)

In the Invecchiare in Chianti (inCHIANTI) study, performed on a cohort from Tuscany, Italy, there was a strong correlation between high levels of lipoprotein (a) and lower-limb PAD in both men and women over 60 years of age.[40] In the MESA study, which included 4 618 participants, significantly elevated levels of lipoprotein (a) were detected only in Hispanic Americans (men and women) with PAD.[41]

High circulating levels of lipoprotein (a), along with other inflammatory markers, were also associated with a reduced ankle–brachial index (ABI) and the presence of clinically significant PAD in an African-American cohort,[42] confirming this is an established risk factor for atherosclerosis, regardless of vascular teritory involved, ethnicity or gender. Its inherent procoagulant effects via its apolipoprotein (a) component, which can inhibit fibrinolysis, makes it an independent marker of acute vascular thrombotic complications.[43]

Interleukin-6 (IL-6)

IL-6 is the main interleukin that exerts procoagulant effects. It is also involved in the inflammatory process by stimulating macrophages and contributing greatly to arterial smooth muscle cell proliferation, thus promoting atherosclerotic plaque formation.[44] Two large studies, the Edinburgh Artery Study[22] and the Walking and Leg Circulation (WALCS) II cohort[44]

demonstrated significantly elevated IL-6 levels, along with higher levels of D-dimer, homocysteine, CRP and soluble vascular cellular adhesion molecule-1 (sVCAM-1) in PAD subjects, which were also related to adverse calf muscle characteristics.[45] This suggests higher levels of inflammation may correlate with functional impairment and functional decline in PAD patients. The association between IL-6 and PAD was stronger in African-American women than in non-Hispanic whites,[42] proving that in addition to gender differences, there are also ethnic dissimilarities.

Tumour necrosis factor-alpha (TNF-α)

TNF-α pro-inflammatory effects range from mediating cellular activation and proliferation to inducing acute-phase responses and destructive cellular outcomes such as apoptosis or cellular necrosis.[46] TNF-α is therefore a pro-angiogenic cytokine.

In one study performed on 91 healthy volunteers, serum TNF-α concentrations were inversely correlated with age, but only in men; in females, no distinct age-related changes were observed.[47] Circulating TNF-α was increased in PAD patients (both men and women) in several studies,[47-49] with additional increases noticed after treadmill stress testing.[48] Gender differences were not assessed[48,49] or were not significant.[50]

Cellular adhesion molecules

Cellular adhesion molecules (CAMs) are integral membrane proteins that mediate cell-to-cell binding. Some of them, such as the selectins and integrins, are involved in leukocyte extravasation and the inflammatory response.[51]

A rise in sICAM and sVCAM-1 levels was associated with the progression of PAD in some studies.[52,53] In both male and female subjects included in the Edinburgh Artery Study, high initial circulating levels of ICAM were correlated with a decrease in the ABI at one-year follow up.[22] Recent research has shown that elevated levels of ICAM-1 were associated with a slower walking pace and a shorter stride length [along with the rise in high-sensitivity CRP (hs-CRP) and reactive oxygen species (ROS) levels], which may suggest that chronic inflammation and oxidative stress can influence walking pace and distance in elderly PAD patients.[23]

McDermott *et al.* demonstrated that high levels of hs-CRP, IL-6, VCAM-1, ICAM-1 and homocysteine were associated with difficulties in completing the six-minute walk test (SMWT) by patients with PAD.[54] In another research published by Gardner *et al.*, African-American women with PAD had higher levels of serum ICAM-1 and leptin than their male counterparts.[3] The same study found that Caucasian women had higher levels of VCAM-1 than men.[3]

Cellular adhesion molecules were also assessed as markers of arterial disease in children. In one paediatric study, ICAM-1 was positively related to CIMT in obese, hypertensive adolescents,[55] while another study performed in healthy young children found that higher plasma levels of the chemokine CCL5, also known as RANTES (regulated on activation, normal T cell expressed and secreted) were positively correlated with arterial stiffness, but not with CIMT.[56] In the same article, monocyte chemo-attractant protein 1 (MCP-1), VCAM and ICAM did not correlate with any of the studied vascular characteristics, namely CIMT, common carotid distensibility or Young's elastic modulus.[56] These conflicting results suggest more research is needed for a better understanding of the role of adhesion molecules in vascular ageing and remodelling.

Markers of oxidative stress

Oxidative stress, a disturbance in the pro-oxidant–antioxidant balance in favour of the former, plays a major role in the development of atherosclerosis[57] and PAD.[58] Numerous studies have shown a rise in ROS,[59,60] alongside a decrease in serum nitric oxide (NO) levels in individuals with PAD.[61] NO (a vasoprotective factor) decrease is partly explained by NO removal secondary to oxidation by excess ROS.[57]

In comparison with controls, PAD subjects (both males and females) had increased urinary isoprostanes (a marker of oxidative stress) and NOX2 (one isoform of NADPH oxidase, a major producer of ROS) activation, contributing to reduced flow-mediated dilatation observed in these patients; these effects were reversed by administration of an antioxidant.[62]

High serum levels of myeloperoxidase, an enzyme involved in inflammation and oxidation,[63] were associated with PAD; the association was stronger in African-Americans compared to non-Hispanic whites, and it was independent of gender.[64] Another study indicated a predictive value for major adverse cardiovascular events, such as myocardial infarction, stroke or death in males or smokers with PAD.[65] In the same way, elevated levels of galectin-3, a lectin involved in inflammation, oxidative stress and angiogenesis, were significantly associated with an increased risk of cardiovascular mortality in PAD subjects.[66]

It seems that endothelial oxidative stress is more important for the development of symptomatic PAD in African-American women, so the effect is gender and race specific. Gardner *et al.* found that African-American females with symptomatic PAD had significantly higher levels of both inflammatory and oxidative stress biomarkers compared to their male counterparts.[3] In the same study, women also had a poorer peripheral circulation than men, especially concerning the smaller distal vessels, with a markedly reduced exercise capacity and daily activity compared to men.[3]

Other markers

Serum beta-2 microglobulin (β2M), one of the major histocompatibility complex class I molecules, is a risk factor for increased CIMT[67] and a predictor of total mortality in older adults.[68] Kals *et al.* had shown that levels of β2M were significantly higher in patients with PAD and correlated with aortic pulse-wave velocity, a measure of arterial stiffness.[69] In a nested case–control study performed in two cohorts, β2M was identified as a risk factor for developing PAD, but only in the male cohort.[70]

Other markers such as leptin and apolipoprotein CIII were found to be higher in women than in men diagnosed with PAD.[3] In a case–control study, adiponectin was significantly lower in women developing PAD compared to healthy controls.[71] Similar to adult data, paediatric observational studies had shown an inverse correlation between plasma adiponectin levels and CIMT,[72] establishing it as a marker of vascular ageing.

Circulating levels of the N-terminal prohormone of brain natriuretic peptide (NT-proBNP), a marker of haemodynamic

stress, were significantly higher in male patients with PAD,[73] but lower than in patients (men and women) with poorly compressible arteries (the latter being defined as having an ABI > 1.4 or an ankle blood pressure > 255 mmHg).[74] Higher NT-proBNP levels were associated with lower functional capacity in individuals with PAD. In this study by Fan and collaborators, female gender was another independent predictor of lower functional capacity.[75] It was also noted that elevated NT-proBNP levels were linearly related to the risk of complications in PAD subjects, including overall cardiovascular mortality, independent of gender.[76] We may speculate that this might be caused by associated heart failure due to concomitant coronary artery disease in these patients, but we cannot completely rule out PAD from the pathophysiological chain.

The soluble receptor for advanced glycation end-products (sRAGE) was found to be lower in patients with coronary artery disease compared to healthy controls.[77] Individuals with associated PAD (82% were males) had even lower levels of sRAGE.[77] sRAGE has protective effects against the harmful interaction of advanced glycation end-products (AGEs) with RAGE.[78] This interaction increases the production of ROS and arterial stiffness,[79] leading to accelerated atherosclerosis and an increased risk of developing symptomatic PAD.

The transforming growth factor-beta (TGF-β) is another cytokine with pro-fibrotic and pro-inflammatory effects.[80,81] Studies showed discordant results regarding TGF-β plasma levels in PAD patients; decreased,[53] increased,[80] or no significant differences[82] versus controls. Gender variations were not reported[53] or not statistically significant.[80,82] These data could be explained by the mixed effects of TGF-β, both as a pro-inflammatory and fibrotic cytokine on the one hand, and as a major orchestrator of vascular repair on the other.

Vascular endothelial growth factors (VEGFs) are essential signalling proteins and key regulators of angiogenesis.[83] In a study in which women and African-Americans were well represented, VEGF-A serum levels were decreased in PAD patients compared to controls.[50] The lower levels of VEGF-A in patients with PAD and claudication suggest that they had lower levels of neo-angiogenesis.[50] Paradoxically, serum VEGF-A levels were increased in several studies on PAD patients.[83-86]

Recent studies demonstrated that there are splice variants of VEGF-A: VEGF-A165a, with pro-angiogenic activity, which was decreased in patients with clinically manifested PAD, and VGFA-A165b, with anti-angiogenic properties, which were increased.[83] This might explain the discrepancies found in studies in which total VEGF-A was measured.

CD163 is a scavenger receptor for the tumour necrosis factor-like weak inducer of apoptosis (TWEAK).[87] TWEAK, a member of the tumour necrosis factor superfamily of structurally related cytokines, determines an increase in pro-inflammatory cytokine secretion, which is associated with the development of atherosclerosis.[88] In one study, performed on white males, the ratio between plasma levels of CD163 and TWEAK was increased in patients with more severe PAD.[89] The results were confirmed in another study performed on both males and females, which did not reveal any gender differences.[90]

Plasma thrombospondin-1 (TSP-1) level, an adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions, was increased in a study that included only white male patients with PAD.[91] Another study, in which 31% of the subjects were females and 62% African-Americans, revealed no significant differences in TSP-1 levels.[92] TSP-1 is a potent inhibitor of angiogenesis by enhancing endothelial colony-forming cell (ECFC) adhesion,[91] and elevated levels may restrict capillary growth in PAD patients,[93] leading to deficient collateral circulation and worsening of PAD symptoms.

Matrix metalloproteinases (MMPs) are a family of endopeptidases that contribute extensively to tissue remodelling by degrading extracellular matrix components.[94] PAD is a manifestation of systemic atherosclerosis and MMPs have been involved in all stages of plaque development.[94] It seems that MMP-9 plays a major role in the process of new blood vessel formation, its deficit compromising ischaemia-induced neovascularisation by decreasing the mobilisation and migration of circulating endothelial progenitor cells (EPC), but also by affecting vasculogenesis.[95] Other results indicate that MMP-10 activity may contribute to plaque rupture and its associated complications.[94]

Recent case–control studies showed an association between PAD and elevated circulating levels of MMP-2,[53,96] MMP-8,[97] MMP-9,[53,96,98] and MMP-10.[96] In some studies, levels of MMPs (MMP-9[96] and MMP-10[94]) were positively correlated with the severity of PAD. These cited works included both males and females, but gender differences were not assessed.

Gardner's study demonstrated that Caucasian women had higher levels of MMP-9, along with higher VCAM-1 and lower hepatocyte growth factor (HGF) levels than Caucasian men. African-American women with clinically manifested PAD also had evidence of increased endothelial oxidative stress compared to their male counterparts.[3] These findings point towards women being a more vulnerable group of PAD subjects, exibiting a more pronounced pro-inflamatory profile of circulating biomarkers than men, and thus requiring stricter lifestyle intervention and medical management.

Novel biomarkers and conventional risk factors

Smoking was for decades considered to be the main risk factor for developing clinically manifested PAD, and recent data confirm its leading pathogenic role along with diabetes mellitus.[12] Cigarette smoking increases oxidative stress at the level of the vascular endothelium and promotes vascular inflammation.[99] In PAD patients, fibrinogen levels are higher as the disease is more extensive, but in female subjects there was a more pronounced increase with smoking status.[19]

Research by Rom *et al.* conducted on a cohort of heavy smokers showed a positive association between CRP levels and smoking history after adjustment for possible confounders.[100] Among over 1 800 participants in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), current smokers had an array of significantly increased inflammatory markers compared to former or non-smokers, ranging from acute-phase proteins (CRP), to chemokines (C-C motif ligands), interleukins and soluble receptors such as soluble vascular endothelial growth factor receptor 3 (sVEGFR-3).[101] However, these values seemed to normalise in time after smoking cessation, approximating those of never smokers after some years. No gender-specific differences were reported in any of these studies. This proves that smoking effects on vascular inflammation are reversible with cessation, and that its consequences are equally harmful in both genders.

Diabetes mellitus (DM), the other major conventional risk factor for PAD, has been shown in multiple research studies to induce a pro-inflammatory state that leads to accelerated atherosclerosis. The proposed mechanism is that of increased expression of adhesion molecules, leading to inflammatory cells crossing the endothelium and forming foam cells, thus initiating and perpetuating the vicious circle of atherosclerotic plaque formation.[102]

A study on patients with diabetic foot showed markedly elevated levels of IL-6 and resistin, coupled with reduced adiponectin plasma levels,[103] suggesting an important anti-inflammatory and anti-atherosclerotic role for this glucose-regulating protein. Tuttle *et al.* reported higher levels of circulating IL-6 and TNF-a in diabetic women, irrespective of clinically manifested cardiovascular disease.[104] Similar results were found in a study on Indian subjects with type 2 DM (which had higher plasma levels of hs-CRP and lower levels of adiponectin compared to healthy controls),[105] confirming the pro-inflammatory state that diabetes mellitus induces, one that transcends gender or age.

Dyslipidaemia has also been related to increased systemic inflammation in several trials. The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study showed that in healthy women with high levels of low-density lipoprotein cholesterol (LDL-C) (> 130 mg/dl; 3.37 mmol/l) and a value of hs-CRP above 2 mg/dl, the overall risk of developing adverse cardiovascular events, even PAD related, increased greatly.[106] Elevated serum levels of CRP in women have been related to the presence of diabetes, the metabolic syndrome and collagen vascular disease,[107] all of these conditions being known for their predisposition to accelerated atherosclerosis.

Multiple other associations between lipid components and inflammatory status have been revealed. For example, in men with established cardiovascular disease, including PAD, a clear association between lipoprotein (a), LDL-C, arterial hypertension and elevated fibrinogen levels has been found,[108] suggesting that the novel biomarkers of atherosclerosis and inflammation are strongly related to traditional risk factors for the disease in a pathophysiological continuum that reveals some gender-specific peculiarities.

Conclusion

Although many of the above biomarkers represent a hallmark of atherogenesis in both genders [lipoprotein (a), TNF-α, sRAGE, VEGF, CD163/TWEAK, thrombospondin-1, galectin-3], some tend to correlate positively and strongly with the presence of PAD in females (CRP, IL-6, ICAM-1, VCAM-1, ROS, leptin, apolipoprotein CIII, adiponectin, MMP-9), while others, such as homocysteine, seem to be associated with the disease only in male individuals. Acknowledging these gender differences could be useful for the early identification and optimal management of patients with PAD.

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