# Biomarkers of inflammation may be of use for identification of more severe peripheral arterial occlusive disease

## *Biomarcadores inflamatórios circulantes podem ser úteis para identificar doença arterial obstrutiva periférica mais grave*

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## Abstract

**Background**: Atherosclerosis is a multifactorial disease with an inflammatory pathophysiological basis. Cytokines released during the atherosclerotic process induce production of C-reactive protein (CRP) in the liver, which is an important marker of inflammation. **Objective**: We tested whether inflammatory biomarkers were associated with deterioration of peripheral arterial occlusive disease (PAOD) in a population at high cardiovascular risk. **Methods**: 1,330 subjects ≥30 years of age underwent clinical and laboratory examinations as part of a population-based study of the prevalence of diabetes. PAOD was defined as an ankle-brachial index (ABI) ≤0.90. After application of exclusion criteria, the sample comprised 1,038 subjects. Traditional risk factors, CRP and interleukin 6 (IL-6) were also compared across three ABI categories (≤0.70; 0.71-0.90; ≥0.90). Mean values for these variables were compared by presence/absence of DAOP (Student's t test) and by ABI categories (ANOVA). Poisson regression and logistic regression models were used to test for associations between risk factors and DAOP and between risk factors and the ABI categories. Pearson's linear correlation coefficients were calculated for the relationship between CRP and IL-6 levels. **Results**: Mean age was 56.8±12.9 years, 54% of the sample were women and the prevalence of DAOP was 21.0% (95%CI 18.4-24.1). Individuals with ABI ≤0.70 had higher concentrations of CRP-us (2.1 vs. 1.8) and of IL-6 (1.25 vs. 1.17). Concentrations of CRP and IL-6 were only correlated in patients with DAOP, (p=0.004). **Conclusions**: The finding that CRP and IL-6 levels were only elevated among people with advanced DAOP may suggest that these biomarkers have a role to play as indicators of more severe disease. Prospective studies are needed to test this hypothesis.

**Keywords:** atherosclerosis; peripheral arterial occlusive disease; biomarkers.

## Resumo

**Contexto**: Aterosclerose é doença multifatorial, cuja base fisiopatológica é um processo inflamatório. Estudos são controversos quanto ao papel dos biomarcadores como fatores de risco. A liberação de citoquinas durante aterogênese promove síntese hepática de proteína C-reativa (PCR), importante marcador inflamatório. **Objetivo**: Avaliamos se biomarcadores inflamatórios estavam associados à deterioração da doença arterial obstrutiva periférica (DAOP), em população de risco cardiovascular. **Métodos**: Estudo populacional sobre prevalência de diabetes, em que 1.330 indivíduos com ≥30 anos foram submetidos a exames clínico-laboratoriais. Diagnóstico de DAOP foi feito pelo índice tornozelo-braço (ITB) ≤0,90. Após exclusões, 1.038 indivíduos foram analisados. Fatores de risco tradicionais, PCR e interleucina 6 (IL-6) foram comparados também segundo três categorias de ITB (≤0,70; 0,71-0,90; ≥0,90). Valores médios das variáveis foram comparados segundo presença de DAOP (teste t Student) e categorias do ITB (ANOVA). Utilizou-se modelo de Poisson e regressão logística para avaliar associações da DAOP e categorias do ITB com fatores de risco. Estimou-se coeficiente de correlação linear de Pearson para relação entre os valores de PCR e IL-6. **Resultados**: A idade média foi 56,8±12,9 anos, 54% mulheres e prevalência de DAOP 21,0% (IC95% 18,4-24,1). Indivíduos com ITB ≤0,70 apresentaram maiores valores de PCR-us (2,1 vs. 1,8) e IL-6 (1,25 vs. 1,17. Apenas em portadores de DAOP, valores de PCR e IL-6 mostraram-se correlacionados (p=0,004). **Conclusão**: O achado de concentrações mais elevadas de PCR e IL-6 apenas em indivíduos com DAOP avançada pode sugerir um papel destes biomarcadores, indicando doença mais grave. Estudos prospectivos são necessários para testar esta hipótese.

**Palavras-chave:** aterosclerose; doença arterial periférica obstrutiva; biomarcadores.

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## INTRODUCTION

Synthesis of C-reactive protein (CRP) in the liver is an innate, nonspecific, immunological defense mechanism[1] that activates the complement system and promotes phagocytosis. To a great extent, synthesis is regulated by interleukin 6 (IL-6), an inflammatory cytokine that is primarily secreted by macrophages and adipocytes.[2] Experimental studies indicate that smooth muscle and endothelial cells of both normal and aneurysmal arteries can also secrete IL-6.[3]

Ultrasensitive assays are capable of detecting discrete increases in blood CRP concentrations (CRP-us) that characterize subclinical chronic inflammation. With relation to atherosclerotic disease, some authors believe that CRP can also be produced in smooth muscle cells of diseased coronary arteries,[4] but this finding is questionable.[5]

The several stages of atherogenesis all involve release of cytokines that stimulate CRP production and so determination of its concentration in blood can be used as an inflammatory marker for monitoring cardiovascular risk.[6] However, a meta-analysis of the role of CRP-us in prediction of cardiovascular risk concluded that although it may be promising, to date no adequately-designed studies of sufficiently long duration have been conducted that could justify its widespread adoption in clinical practice.[7]

An initial trigger of formation of fatty streaks is endothelial dysfunction, which can be precipitated by smoking, arterial hypertension, hyperglycemia, hypercholesterolemia, hyperhomocysteinemia and even infections. Initial steps in atherosclerosis include expression of adhesion molecules and transmigration of monocytes into the subendothelial space, where they absorb lipids from foam cells. These cells secrete inflammatory mediators, including IL-6 which acts to reduce de lipoprotein lipase activity, stimulating phagocytosis of lipids further still. There is evidence to indicate that CRP and IL-6 amplify and perpetuate the inflammatory response in atheroma.[8]

In terms of the different sites affected by atherosclerosis, peripheral arterial occlusive disease (PAOD) is one of the most important conditions because it is becoming ever more prevalent in modern society, as a result of increasing life expectancy. It is estimated that 202 million people had PAOD worldwide in 2010. Over the last ten years, PAOD prevalence in countries with low to medium per capita income increased by 28.7%, and its prevalence in high income countries increased by 13.1%.[9] The growing interest in early diagnosis of PAOD is not only the result of its increasing prevalence, but also because it is related to diseases in other parts of the body, such as coronary or cerebral conditions.[10] In view of this, identification of the disease itself and also of indicators of its severity is desirable for the purposes of planning interventions.

While there is evidence that elevated CRP and IL-6 concentrations are indicative of the subclinical inflammatory processes present in atherosclerotic cardiovascular disease, there is no consensus on the etiopathogenic role that these substances play in arterial injury, and this is particularly true of investigations into PAOD.

This research was conducted in order to investigate whether CRP and IL-6 concentrations were related to different classes of PAOD, by means of a population study of Japanese-Brazilian subjects, who are a population group that is known to have high cardiovascular risk.[11-13]

## METHODS

This analysis was conducted with a population of Japanese-Brazilians living in Bauru, SP, Brazil. The sample included both sexes and the minimum age was 30 years. Details of the umbrella study into the prevalence of diabetes and metabolic syndrome have been described elsewhere.[11-13] The study was approved by the Institutional Ethics Committee and free and informed consent forms were signed by all participants. A total of 1,330 people underwent a clinical examination, including anthropometric measurements, arterial blood pressure measurement and calculation of the Doppler ankle-brachial index (ABI). Additionally, blood samples were taken after 12 hours' fasting. The exclusion criteria were missing clinical or laboratory data preventing analysis (255 participants), ABI >1.40 (one participant) and CRP-us concentration >10 mg/L (36 participants). As a result, 1,038 people were enrolled on the study.

Weight and height were measured with subjects unshod and wearing minimal clothing and the results used to calculate body mass index (BMI). Waist circumference was measured using an inextensible tape measure, at the midpoint between the last floating rib and the iliac crest along a plane parallel to the floor. Hip circumference was measured at the height of the buttocks, passing over the pubic symphysis. The waist-hip ratio was calculated by dividing waist circumference by hip circumference.

Since this is a population with Asian origins, the BMI and waist circumference cutoffs used for diagnosis of obesity and central obesity were those

recommended by the Japan Society for the Study of Obesity (JASO)[14] and the waist-hip ratio cutoff used was that recommended by the World Health Organization (WHO).[15]

Arterial blood pressure was measured using an automatic blood pressure meter (Omron HEM-712C, Omron Healthcare, USA), sitting down, after 5 minutes at rest. The figures used for analysis were the arithmetic means of the last two systolic and diastolic pressure readings. Subjects with pressures greater than 140×90 mmHg and those that were on medication for high blood pressure were diagnosed as having arterial hypertension.[16]

Participants with capillary glycemia greater than 200 mg/dL after 12 hours' fast were not subjected to the oral glucose tolerance test. Venous blood samples were taken after fasting and 2 hours after intake of 75 grams of glucose. Subjects were allocated to glucose tolerance categories in accordance to American Diabetes Association criteria.[17] Therefore, participants with fasting glycemia <100 mg/dL or post-challenge glycemia <140 mg/dL were considered normoglycemic. Subjects with fasting glycemia greater than or equal to 100 mg/dL, but less than 126 mg/dL, and with glycemia 2 hours after challenge below 140 mg/dL were defined as having abnormal fasting glycemia (AFG). Impaired glucose tolerance (IGT) was defined as fasting glycemia greater than or equal to 100 mg/dL and post-challenge glycemia from 140 to 199 mg/dL. Subjects were diagnosed as diabetic if they exhibited fasting glycemia greater than or equal to 126 mg/dL or post-challenge glycemia greater than or equal to 200 mg/dL, or if they were already on hypoglycemic drugs.

The reference values used to diagnose dyslipidemias were those recommended by the NCEP at the time of enrolment.[18] Participants were considered to have normal lipid profiles if total cholesterol was ≤200 mg/dL, LDL-cholesterol was ≤130 mg/dL, HDL-cholesterol was ≥35 mg/dL for men or ≥45 mg/dL for women and triglycerides were ≤200 mg/dL. Subjects were defined as having a dyslipidemia if any of these variables were outside of these limits.

Uric acid was considered normal up to 6 mg/dL for women or up to 7 mg/dL for men.[19] Homocysteine was assayed using high performance liquid chromatography. Homocysteine results were considered normal up to 15 mmol/L.[20] Chemiluminescence was used to determine CRP-us and IL-6 concentrations (Immulite, Diagnostic Products Corporation, USA).

### Diagnosis of PAOD

Peripheral arterial occlusive disease was diagnosed using an 8 mHz continuous wave Doppler machine by Imbracios®. The ABI was calculated by dividing the pressure at the arteries in the ankle by the greatest pressure measured at the brachial arteries. Indices of ≤0.90 or >1.40 were considered abnormal, as recommended by the TASC II (Transatlantic Society Consensus).[21] The sample was also analyzed after stratification by ABI into the following three categories: ≤0.70; 0.71 to 0.90; and >0.90.[22]

### Statistical analysis

Variables were expressed as percentages, means and standard deviations. The sample was stratified by sex or according to PAOD, diagnosed according to ABI results.

Crude analysis was used to identify associations between variables using the chi-square test and prevalence ratios (PR) were estimated for points and for 95% confidence intervals. Mean values for variables were compared according to presence or absence of PAOD or according to ABI categories (≤0.70; 0.71-0.90; >0.90) using Student's t test or analysis of variance (ANOVA) with Bonferroni's correction, respectively.

Poisson regression models were used to obtain PR of PAOD by cardiovascular risk factors. The initial model began with all variables that had been associated with PAOD ($p<0.150$) in the crude analysis and then variables that could be removed without changing the model's predictive capacity were eliminated one-by-one. A similar procedure was employed to obtain odds ratios in an ordered logistic regression model, by ABI categories (≤0.70; 0.71-0.90; >0.90). Pearson's linear correlation coefficients were calculated to investigate possible relationships between CRP and IL-6 values. This analysis was supplemented by comparison of mean IL-6 concentrations across CRP-us terciles using ANOVA. Results where $P<0.05$ were considered significant.

Stata 8.0 (Statacorp, 2004, Stata statistical software release 7.0 College Station, TX Stata Corporation) was employed for these analyses.

## RESULTS

The 1,038 participants (54% female) had a mean age of 56.8 (±12.9) years. Men had higher mean values for BMI, waist-hip ratio, arterial blood pressure, fasting glycemia, triglycerides, uric acid, homocysteine and IL-6, while women had higher

total cholesterol, LDL-cholesterol, HDL-cholesterol and CRP-us concentrations (Table 1).

Prevalence rates for risk factors stratified by sex are shown in Table 2. Prevalence rates were higher among men for diabetes, smoking, hypertriglyceridemia, hyperuricemia, hyperhomocysteinemia, low HDL-cholesterol and elevated IL-6 concentration, while elevated LDL-cholesterol and CRP-us were more frequent among the women. Anthropometry results showed that elevated BMI was more frequent among the men, while abdominal obesity was more prevalent among the women (Table 2).

The prevalence of PAOD was 21.1% (95%CI 18.4-24.1), with no difference between the sexes (19.2% vs. 22.7%, p>0.05). Since clinical variables for subjects with and without PAOD behaved similarly when broken down by sex, these results are shown for both sexes together. Individuals with PAOD were, on average, older than those free from the disease (60.0 vs. 56.0 years, p<0.001) and had higher results for systolic arterial blood pressure and homocysteine, but not for CRP-us or IL-6 (data not shown in table). Higher prevalence rates of PAOD were therefore observed in the older age range, among hypertense subjects and in those with hyperhomocysteinemia. No differences were detected between strata with and without PAOD in any of the other variables (Table 3).

After adjustment of the PAOD prevalence ratios for the variables analyzed, only smoking and arterial hypertension remained independently associated with the disease (Table 4).

These associations were unchanged by stratification into three ABI categories (data not shown in tables).

Analyzing individuals with ABI ≤0.70 in isolation, this subset was older, smoked a higher number of cigarettes/day and had higher mean CRP-us and IL-6. Furthermore, these subjects had higher mean systolic pressure, fasting glycemia, 2 hour post-challenge glycemia, triglycerides and homocysteine (Table 5). Eighty-five percent of these individuals with ABI ≤0.70 were hypertense and 70% had diabetes and high waist-hip ratios (data not shown in tables).

Figures 1 and 2 illustrate the relationships between CRP-us and IL-6 for individuals with and without PAOD, respectively, in the form of scatter plots. Significant correlations between these variables were only detected for subjects with PAOD (r=0.24, p=0.010). Stratification of CRP-us concentrations into terciles (Figures 3 and 4) only revealed significant differences between mean IL-6 concentrations for individuals with PAOD.

## DISCUSSION

In this study of a population of Japanese-Brazilians, a relatively high prevalence of PAOD (21.1%) was expected, considering the unfavorable cardiometabolic profile that has been described previously.[11-13,23] Although mean values and frequencies of risk factors among men were higher, the prevalence of PAOD was similar for both sexes. Similar figures for PAOD have been observed in other populations at high cardiovascular risk. The

**Table 1.** Means (SD) of demographic, anthropometric, clinical and biochemical variables, by sex.

| | Men N=473 | Women N=565 | Total N=1038 | p |
|---|---|---|---|---|
| Age (years) | 56.7 (12.9) | 56.9(12.4) | 56.8(12.6) | 0.364 |
| Cigarettes per day* | 18.1 (7.5) | 16.8(7.7) | 17.7(7.6) | 0.138 |
| Body mass index (kg/m2) | 25.2 (3.8) | 24.5(3.8) | 24.8(3.8) | 0.001 |
| Waist-hip ratio | 0.91 (0.06) | 0.84(0.07) | 0.88(0.08) | <0.001 |
| Diastolic BP (mmHg) | 81 (13) | 77(13) | 79(13) | <0.001 |
| Systolic BP (mmHg) | 135 (23) | 132(25) | 133(24) | 0.006 |
| Fasting glycemia (mg/dL)* | 127 (31) | 122 (37) | 124 (34) | <0.001 |
| 2hr post-challenge glycemia (mg/dL)* | 168 (86) | 160(69) | 164(77) | 0.169 |
| Total cholesterol (mg/dL) | 212 (42) | 217 (43) | 215 (43) | 0.030 |
| Triglycerides (mg/dL)* | 279 (244) | 199 (139) | 232(197) | <0.001 |
| HDL-cholesterol (mg/dL)* | 49 (12) | 52 (10) | 51 (11) | <0.001 |
| LDL-cholesterol (mg/dL) | 127 (38) | 133 (380) | 130 (38) | 0.009 |
| Homocysteine (mg/dL)* | 13.1 (7.5) | 9.9 (4.1) | 11.4 (6.1) | <0.001 |
| C-reactive protein (mg/L)* | 1.6 (1.7) | 1.9 (1.8) | 1.8 (1.8) | 0.015 |
| Uric acid (mg/L)* | 7.0 (1.8) | 5.3 (1.3) | 6.1 (1.8) | <0.001 |
| IL-6 (pg/dL)* | 1.19 (0.94) | 1.03 (1.08) | 1.10 (1.02) | 0.003 |

*Figures log-transformed for analysis.

**Table 2**. Prevalence rates of cardiovascular risk factors in a Japanese-Brazilian population, by sex.

| | | Male | | Female | | p-value |
|---|---|---|---|---|---|---|
| | | N | % | N | % | |
| Age | ≤60 years | 284 | 60.0 | 335 | 59.3 | 0.806 |
| | <60 years | 189 | 40.0 | 230 | 40.7 | |
| Smoking | No | 218 | 46.2 | 499 | 88.8 | <0.001 |
| | Yes (in past) | 91 | 19.3 | 39 | 6.9 | |
| | Yes (current) | 163 | 34.5 | 24 | 4.3 | |
| Central obesity | No | 350 | 74.3 | 153 | 27.1 | <0.001 |
| | Yes | 121 | 25.7 | 411 | 72.9 | |
| BMI (kg/m2) | <23 | 131 | 27.8 | 213 | 37.7 | 0.001 |
| | 23.0 to 24.9 | 107 | 22.6 | 130 | 23.0 | |
| | ≥25 | 234 | 49.6 | 222 | 39.3 | |
| Arterial hypertension | No | 250 | 52.8 | 317 | 56.1 | 0.295 |
| | Yes | 223 | 47.2 | 248 | 43.9 | |
| Glucose tolerance | Normal | 16 | 3.4 | 42 | 7.5 | 0.008 |
| | AFG | 170 | 35.9 | 207 | 36.7 | |
| | IGT | 104 | 22.0 | 136 | 24.1 | |
| | DM | 183 | 38.7 | 179 | 31.7 | |
| Hypercholesterolemia | No | 189 | 40.0 | 203 | 35.9 | 0.182 |
| | Yes | 284 | 60.0 | 362 | 64.1 | |
| Low HDL | No | 395 | 83.5 | 514 | 91.0 | <0.001 |
| High LDL | Yes | 78 | 16.5 | 51 | 9.0 | 0.002 |
| | No | 269 | 56.9 | 267 | 47.3 | |
| | Yes | 204 | 43.1 | 298 | 52.7 | |
| Hypertriglyceridemia | No | 131 | 27.7 | 236 | 41.8 | <0.001 |
| | Yes | 342 | 72.3 | 329 | 58.2 | |
| Homocysteine | ≤15 mg/dL | 286 | 77.3 | 414 | 92.2 | <0.001 |
| | >15 mg/dL | 84 | 22.7 | 35 | 7.8 | |
| Hyperuricemia | No | 216 | 45.7 | 139 | 24.6 | <0.001 |
| | Yes | 257 | 54.3 | 426 | 75.4 | |
| CRP-us (mg/L) | 0.00-0.07 | 184 | 38.9 | 197 | 34.9 | 0.044 |
| | 0.08-0.18 | 163 | 34.5 | 177 | 31.3 | |
| | 0.19-0.99 | 126 | 26.6 | 191 | 33.8 | |
| IL6 (pg/dL) | 0.00-0.73 | 33 | 25.0 | 66 | 42.0 | 0.005 |
| | 0.74-1.10 | 42 | 31.8 | 46 | 29.3 | |
| | 1.10-9.3 | 57 | 43.2 | 45 | 28.7 | |

US PARTNERS program was designed to study the prevalence of PAOD, among other cardiovascular diseases, finding a 29% prevalence of PAOD among individuals aged >70 years or aged >50 years with comorbidities (diabetes and smoking).[24] The POPADAD study assessed 8,000 diabetic people aged ≥40 years and found a PAOD prevalence of 20.1%.[25]

In our setting, a multicenter study into the prevalence of PAOD among the general population (n=1,170) of 72 urban centers in Brazil found a prevalence of just 10.5%. It is worth noting that the age range was lower (≥18 years) and the sample was more representative of the Brazilian population because it was not comprised of genetically homogenous subjects nor of people at high cardiovascular risk.[26] Results published previously by our research team have shown that Japanese-Brazilians are a high-risk group, in terms of their figures for obesity, diabetes mellitus, arterial hypertension and dyslipidemia.[11-13,23]

We detected significant associations between PAOD and two classical risk factors: smoking and arterial hypertension. Subjects with more advanced forms of the disease (ABI ≤0.70) had higher values for the (not classical) biomarkers investigated (CRP-us and IL-6). This group of Japanese-Brazilians had the worst cardiometabolic profile. Although this subset contained a small number of individuals (n=20), it was still possible to detect statistically significant differences. It should be emphasized that

**Table 3.** Number, percentage and prevalence ratios (95% confidence intervals) for categories of demographic and clinical variables from Japanese-Brazilians, stratified by presence or absence of peripheral arterial occlusive disease (PAOD).

| | | Has PAOD n=219 | | Free from PAOD n=819 | | Total n=1038 | | Chi-square | PR | 95%CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | | | |
| Sex | Female | 128 | 22.7 | 437 | 77.3 | 565 | 100 | 1.80 | 1 | |
| | Male | 91 | 19.2 | 382 | 80.8 | 473 | 100 | | 0.85 | 0.67-1.08 |
| Age | ≤60 years | 110 | 17.8 | 509 | 82.2 | 619 | 100 | 10.2 | 1 | |
| | >60 years | 109 | 26.0 | 310 | 74.0 | 419 | 100 | | 1.46 | 1.16-1.85 |
| Smoking | No | 150 | 20.9 | 567 | 79.1 | 717 | 100 | 0.39 | 1 | |
| | Yes (in past) | 38 | 20.3 | 149 | 79.7 | 187 | 100 | | 0.97 | 0.71-1.33 |
| | Yes (current) | 30 | 23.1 | 100 | 76.9 | 130 | 100 | | 1.10 | 0.78-1.56 |
| High waist-hip ratio | No | 101 | 20.1 | 402 | 79.9 | 503 | 100 | 0.57 | 1 | |
| | Yes | 117 | 22.0 | 415 | 78.0 | 532 | 100 | | 1.10 | 0.86-1.39 |
| Body mass index (kg/m2) | <23 | 76 | 22.1 | 268 | 77.9 | 344 | 100 | 3.10 | 1 | |
| | 23.0 to 24.9 | 57 | 24.1 | 180 | 75.9 | 237 | 100 | | 1.09 | 0.81-1.47 |
| | ≥25 | 85 | 18.6 | 371 | 81.4 | 456 | 100 | | 0.84 | 0.64-1.11 |
| Arterial hypertension | No | 101 | 17.8 | 466 | 82.2 | 567 | 100 | 8.1 | 1 | |
| | Yes | 118 | 25.1 | 353 | 74.9 | 471 | 100 | | 1.41 | 1.11-1.78 |
| Glucose tolerance | Normal | 8 | 13.8 | 50 | 86.2 | 58 | 100 | 4.40 | 1 | |
| | AFG | 73 | 19.4 | 304 | 80.6 | 377 | 100 | | 1.40 | 0.71-2.76 |
| | IGT | 59 | 24.6 | 181 | 75.4 | 240 | 100 | | 1.78 | 0.90-3.52 |
| | DM | 79 | 21.8 | 283 | 78.1 | 362 | 100 | | 1.58 | 0.81-3.10 |
| Hypercholesterolemia | No | 81 | 20.7 | 311 | 79.3 | 392 | 100 | 0.07 | 1 | |
| | Yes | 138 | 21.4 | 508 | 78.6 | 646 | 100 | | 1.03 | 0.81-1.32 |
| Low HDL | No | 190 | 20.9 | 719 | 79.1 | 909 | 100 | 0.17 | 1 | |
| | Yes | 29 | 22.5 | 100 | 77.5 | 129 | 100 | | 1.08 | 0.72-1.52 |
| High LDL | No | 112 | 20.9 | 424 | 79.1 | 536 | 100 | 0.03 | 1 | |
| | Yes | 107 | 21.3 | 395 | 78.7 | 502 | 100 | | 1.02 | 0.81-1.29 |
| Hypertriglyceridemia | No | 76 | 20.7 | 291 | 79.3 | 367 | 100 | 0.05 | 1 | |
| | Yes | 143 | 21.3 | 528 | 78.7 | 671 | 100 | | 1.03 | 0.80-1.32 |
| CRP-us# (mg/L) | 0.00-0.07 | 72 | 18.9 | 309 | 81.1 | 381 | 100 | 0.38 | 1 | |
| | 0.08-0.18 | 62 | 18.2 | 279 | 81.8 | 341 | 100 | | 0.96 | 0.71-1.31 |
| | 0.19-0.99 | 64 | 20.1 | 255 | 79.9 | 319 | 100 | | 1.06 | 0.78-1.44 |
| Homocysteine (mg/dL) | ≤15 | 140 | 20.0 | 560 | 80.0 | 700 | 100 | 5.36 | 1 | |
| IL-6#(pg/dL) | >15 | 35 | 29.4 | 84 | 70.6 | 119 | 100 | 2.59 | 1.47 | 1.07-2.02 |
| | 0.00-0.73 | 35 | 35.4 | 64 | 64.6 | 99 | 100 | | 1 | 0.93-1.87 |
| | 0.74-1.10 | 41 | 46.6 | 47 | 53.4 | 88 | 100 | | 1.32 | 0.86-1.73 |
| | 1.10-9.3 | 44 | 43.1 | 58 | 56.9 | 102 | 100 | | 1.22 | |

#Figures log-transformed for statistical tests; PR: prevalence ratio; 95%CI: 95% confidence interval.

the significant correlation between CRP and IL-6 values was only detected among subjects who did have PAOD (Figure 2). The significant increase in IL-6 concentrations in CRP terciles was also in line with this finding (Figure 4).

Tzoulaki et al. investigated 1592 smokers aged 55 to 74 years and found a 23% prevalence of asymptomatic PAOD, observing that IL-6 increased in proportion to reductions in ABI over follow-up periods ranging from 5 to 12 years.[27] Their findings are compatible with the hypothesis that the proinflammatory state is exacerbated in proportion to the degree to which the disease worsens. Similarly, we also found a relationship between PAOD severity and inflammatory marker levels. However, it is known that smoking affects IL-6 and CRP levels.[28] Both IL-6 and CRP levels are associated with cumulative tobacco use (years/packs) and, among ex-smokers, with time free from smoking.[29] Signorelli et al. also observed elevated IL-6 levels in 20 non-smoking patients with a mean ABI of 0.72 ($p<0.001$).[30]

The same findings have been described by Danielsson et al. after analyzing five groups of

people: patients with intermittent claudication and critical ischemia, both with and without diabetes, and a control group. An analysis adjusted for multiple factors only detected a relationship between inflammatory markers (IL-6 and CRP-us) and critical ischemia. These authors concluded that the relationship is dependent on the severity of PAOD, irrespective of the presence of other risk factors.[31]

The relationship between inflammatory markers and PAOD severity has also been investigated in the

**Table 4.** Prevalence ratios (PR) for PAOD and respective 95% confidence intervals (95%CI) in a population of Japanese-Brazilians, by variables selected for models (initial and final models).

| Variable | | Initial model | | Adjusted model | |
|---|---|---|---|---|---|
| | | PR | 95%CI | PR | 95%CI |
| Sex | Female | 1 | | 1 | |
| | Male | 0.66 | 0.42-1.02 | 0.66 | 0.44-1.01 |
| Age | ≤60 years | 1 | | 1 | |
| | >60 years | 0.98 | 0.71-1.36 | 0.94 | 0.69-1.23 |
| Smoking | No | 1 | | 1 | |
| | Yes (past) | 1.45 | 0.91-2.31 | 1.44 | 0.92-2.33 |
| | Yes (current) | 2.14 | 1.32-3.50 | 2.16 | 1.34-3.48 |
| Arterial hypertension | No | 1 | | 1 | |
| | Yes | 1.61 | 1.12-2.31 | 1.56 | 1.12-2.22 |
| Glucose tolerance | Normal | 1 | | | |
| | AFG | 0.67 | 0.38-1.21 | 0.71 | 0.40-1.32 |
| | IGT | 0.76 | 0.42-1.38 | 0.65 | 0.43-1.44 |
| | DM | 0.66 | 0.37-1.17 | 0.65 | 0.39-1.23 |
| Homocysteine (mg/dL) | ≤15 | 1 | | 1 | |
| | >15 | 1.25 | 0.88-1.77 | 1.26 | 0.89-1.78 |
| CRP-us# (mg/L) | 0.00-0.07 | 1 | | 1 | |
| | 0.08-0.18 | 0.62 | 0.35-1.10 | 0.77 | 0.52-1.14 |
| IL-6# (pg/dL) | 0.19-0.99 | 0.72 | 0.40-1.30 | 0.97 | 0.66-1.41 |
| | 0.00-0.73 | 1 | 0.70-2.26 | 1 | 0.76-1.05 |
| | 0.74-1.10 | 1.26 | | 0.88 | |
| | 1.10-9.3 | 1.09 | 0.60-1.98 | 0.91 | 0.88-1.18 |

#Figures log-transformed for analysis.

**Table 5.** Means and standard deviations (SD) for demographic, anthropometric and clinical variables from a Japanese-Brazilian population, by ankle-brachial index categories.

| Variable | Ankle-brachial index | | | F statistic | P |
|---|---|---|---|---|---|
| | ≤0.70 | 0.71-0.90 | >0.90 | (Anova) | |
| Age (years) | 65.1 (12.7) | 59.3 (13.2) | 56.0 (12.3) | 10.14 | < 0.001 |
| Number of cigarettes/day* | 22.5 (6.1) | 17.9 (6.7) | 17.3 (7.8) | 1.99 | 0.141 |
| Waist circumference (cm) | 86.0 (10.3) | 82.5 (9.4) | 84.2 (10.5) | 2.56 | 0.078 |
| Waist-hip ratio | 0.91 (0.08) | 0.87 (0.07) | 0.88 (0.08) | 3.06 | 0.047 |
| BMI (kg/m2) | 25.2 (4.8) | 24.4 (3.7) | 24.9 (3.8) | 1.34 | 0.263 |
| Diastolic BP (mmHg) | 85.2 (11.7) | 79.7 (13.9) | 79.1 (13.2) | 2.09 | 0.125 |
| Systolic BP (mmHg) | 151.3 (23.0) | 137.6 (27.5) | 1312.0 (23.4) | 9.86 | < 0.001 |
| Fasting glycemia (mg/dL)* | 148.0 (49.1) | 120.5 (28.3) | 124.9 (35.2) | 6.56 | 0.002 |
| 2hr post-challenge glycemia (mg/dL)* | 239.2 (115.9) | 164.1 (70.4) | 162.2 (77.0) | 7.45 | 0.001 |
| Total cholesterol (mg/dL) | 209.3 (31.2) | 214.3 (40.8) | 214.8 (43.1) | 0.17 | 0.840 |
| HDL (mg/dL)* | 49.5 (10.8) | 50.4 (10.2) | 51.4 (11.7) | 0.65 | 0.523 |
| LDL (mg/dL) | 126.1 (26.5) | 130.0 (38.8) | 130.6 (38.1) | 0.15 | 0.864 |
| Triglycerides (mg/dL)* | 282.0 (226.1) | 227.3 (185.1) | 231.4 (199.7) | 1.09 | 0.336 |
| CRP*(mg/L) | 0.21 (0.23) | 0.18 (0.16) | 0.18 (0.18) | 0.21 | 0.813 |
| IL6*(pg/dL) | 1.25 (0.64) | 0.98 (0.50) | 1.17 (1.27) | 0.74 | 0.478 |
| Homocysteine* | 12.3 (5.8) | 12.2 (6.5) | 11.1 (6.0) | 3.48 | 0.031 |

*Figures log-transformed for analysis.

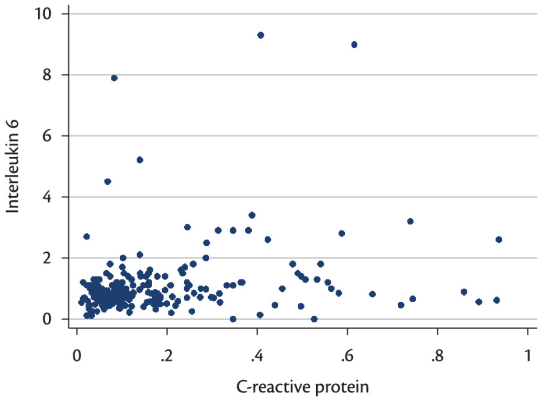


**Figure 1**. Correlation between CRP and IL-6 levels in people without PAOD. r=0.04 (p=0.624).

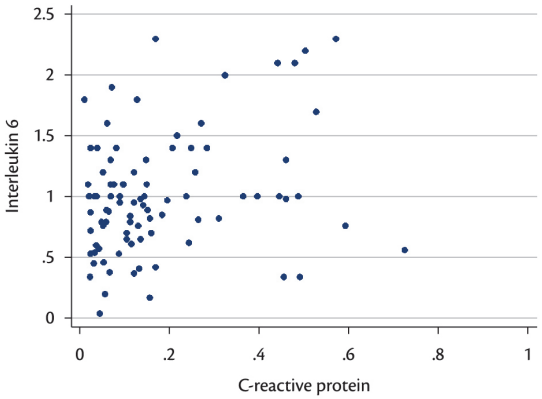


**Figure 2**. Correlation between CRP and IL-6 levels in people with PAOD. r=0.24 (p=0.010).

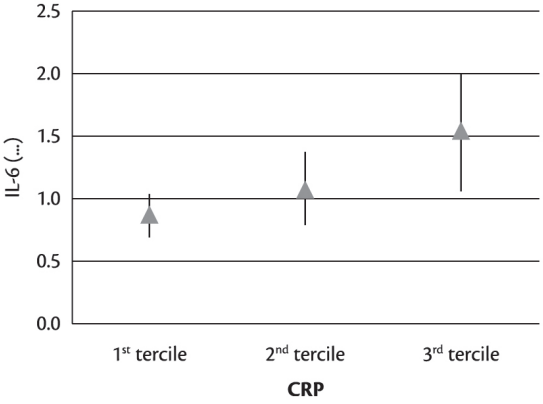


**Figure 3.** IL-6 levels by CRP terciles for people without PAOD. (p=0.658).

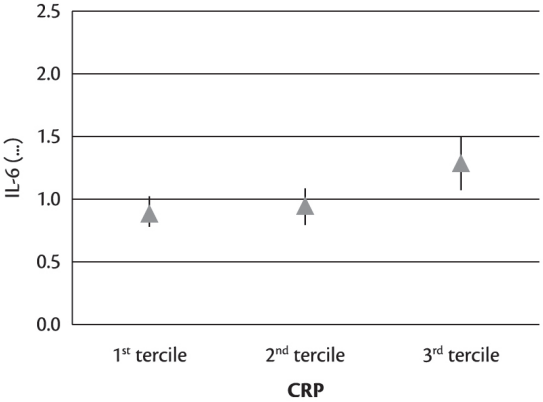


**Figure 4.** IL-6 levels by CRP terciles for people with PAOD. (p=0.004).

carotid territory. No association was detected between IL-6 or CRP-us and subclinical atherosclerosis assessed in 1,111 individuals with carotid myointimal thickening,[32] which is similar to our findings since we also failed to detect a relationship with inflammatory markers in patients with PAOD in the initial phases. However, the study did detect a positive relationship between carotid stenosis (≥25%) and CRP-us. These authors also failed to detect any relationship between the marker and myointimal hyperplasia of the carotid, suggesting that CRP-us is a marker of more advanced disease.[33]

The same findings have been observed in the coronary territory. A case-control study of 926 men aged 50 to 59 found that among subjects who suffered infarction or sudden death over a 5-year follow-up period had had elevated IL6 and CRP levels at the start of the study. Patients who only exhibited angina during this period had had normal levels of these markers at the study outset. Additionally, the relative risk of infarction and death increased in line with increases in IL6 levels.[34]

Another prospective study, analyzing the association between IL-6 and mortality in 718 patients with coronary disease, found that serum IL-6 concentrations of patients who died from coronary disease during a 2.3-year follow-up period were significantly higher at the study outset. Interleukin 6 levels were associated with cardiovascular mortality, conferring a relative risk of 2.04 (95%CI 1.34-3.68). These findings support the hypothesis that a subclinical chronic inflammatory process plays a role in determining the outcome of atherosclerotic disease and is a sign of patients with poor prognosis.[35] Biasucci et al. have published further evidence in support of this theory, showing that IL-6 levels increased after admission in patients with unstable angina who went on to develop complications.[36]

Our findings are in agreement with the literature reviewed. Both IL-6 and CRP-us concentrations

appear to reflect the intensity of occult inflammation in the atherosclerotic plaque and may be capable of determining its vulnerability to rupture and, consequently, of indicating disease severity.[37]

Although the cross-sectional design has inherent limitations that prevent conclusions on cause-effect relationships, the strengths of our study lie in its populational characteristic and in the uniformity of the population investigated.

In summary, our data suggest the existence of a relationship between atherosclerosis of peripheral arteries and inflammatory markers and show that these markers can indicate disease severity. Patients with advanced PAOD may have few or even no symptoms if they engage in little or no activity, as is the case with bedridden patients. The same may be true of atherosclerotic disease in the cerebral and coronary territories. The ability to detect these patients with more advanced forms of the disease would be of great value and the hypothesis merits testing in prospective studies, since this information would facilitate the tasks of mapping those patients with least favorable atherosclerotic disease prognoses and of planning more aggressive treatments.

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