Significance of Antinuclear Antibodies and Rheumatoid Factor in Patients with Advanced Peripheral Arterial Disease

Laila R. Qadan¹ Adel A. Ahmed² Suhair Abdel-Jalil³ Marzouk A. Al-Bader⁴

Departments of ¹Medicine-Endocrinology and ²Radiology, Faculty of Medicine, Kuwait University, and ³Department of Surgery, Mubarak Al-Kabeer Hospital, Ministry of Health, Kuwait; ⁴Department of Rheumatology, St. Francis Hospital/University of Illinois Affiliate, Chicago, Ill., USA

Key Words
Antinuclear antibodies · Rheumatoid factor · Peripheral arterial disease · C-reactive protein · Metabolic syndrome

Abstract
Objective: To determine whether or not elevated titers of antinuclear antibodies (ANA) and/or rheumatoid factor (RF) are associated with patients with advanced peripheral arterial disease (PAD). Subjects and Methods: A cross-sectional study was done between September 2005 and December 2006. Fifty-eight patients with clinical and angiographic evidence of PAD and 41 controls were studied. Controls had no documented history of peripheral, coronary or cerebral vascular disease. All subjects were screened for metabolic syndrome and C-reactive protein (CRP) as risk factors for peripheral vascular disease. Additionally, all were tested for antimitochondrial, anti-neutrophil cytoplasmic and anti-smooth muscle antibodies; those with positive results were excluded. ANA and RF were measured in sera from cases and controls.

Results: One case and 3 controls had positive anti-smooth muscle antibodies and were therefore excluded from statistical analysis. Metabolic syndrome was significantly more prevalent in patients than controls (p < 0.05). Mean CRP level was 4.78 ± 7.70 and 2.65 ± 3.86 mg/dl in cases and controls, respectively (p = 0.021). ANA were detected at a titer of ≥1:40 in 6 (10.5%) of the advanced PAD patients but none of the controls; the difference was not statistically significant. RF was less prevalent in cases than controls (p < 0.05).

Conclusion: RF and ANA do not appear to be associated with PAD in a Kuwaiti population.

Introduction

Atherosclerosis is an inflammatory process of multifactorial origin which results in vascular occlusion and presents as coronary, cerebral and peripheral arterial disease (PAD). Metabolic syndrome and C-reactive protein (CRP) are risk factors for its development and progression [1–5].

Autoimmunity has been thought to be a causative factor for atherosclerosis. In fact, it seems that both cellular and humoral immune systems are involved in the development and progression of atherosclerosis [6]. The reports of autoantibodies and the chronic inflammatory response associated with it are far from being conclusive. Autoantibodies, which have been implicated to be associated with atherosclerosis, include those against oxidized...
low-density lipoprotein, cardiolipin, β2-glycoprotein 1, heat shock proteins 60/65, antinuclear antibodies (ANA) and rheumatoid factor (RF) [6–9].

Subjects with rheumatoid arthritis and systemic lupus erythematosus have increased risk of coronary atherosclerosis [10–15]. It was described that up to 70% of patients with severe coronary artery disease have increased ANA [8], and RF was suggested to be an independent risk factor for ischemic heart disease in men [9].

Although RF and ANA are strongly associated with rheumatoid arthritis and systemic lupus erythematosus, respectively, they are not highly specific for these diseases. Therefore, studies [8, 9] were conducted to delineate an association between those antibodies and athrogenesis. We further hypothesize that if ANA and RF have a pathogenic role in coronary atherosclerosis, we would find a similar positive association in patients with advanced PAD. Hence, the objective of this study was to determine whether or not ANA and RF are associated with advanced PAD.

**Subjects and Methods**

A cross-sectional study was carried out in Kuwait from September 2005 to December 2006. The study was conducted in accordance with the ethical rules and regulations of Kuwait’s University Review Board for evaluating human research. Fifty-eight patients with clinically advanced and angiographically proven PAD and 41 controls were initially enrolled. Patients were originally evaluated by vascular surgeons and were referred for angiography secondary to failure of conservative medical therapy. These patients had either disabling claudication or critical leg ischemia. Disabling claudication referred not only to claudication distance but interference with everyday living requirements and preferences. Critical limb ischemia is the term used to delineate those patients whose arterial disease has resulted in a breakdown of the skin (ulcer or gangrene) or pain in the foot even at rest; it therefore coincides with the Rutherford 4, 5, and 6 categories of the new SVS-ISCVS recommendation of reporting standards [16]. All control subjects had no clinical history of peripheral, coronary or cerebral arterial disease.

Patients and controls had to undergo a standardized diagnostic protocol including waist circumference, systolic and diastolic blood pressure. Laboratory tests were performed to determine the lipid profile (serum triglycerides, serum HDL cholesterol), fasting blood glucose and CRP. The data were used to classify them for the absence or presence of metabolic syndrome. Metabolic syndrome was defined using the modified National Cholesterol Education Program, Adult Treatment Panel III NCEP-ATP III criteria [10 –15]. It was described that up to 70% of patients with severe coronary artery disease have increased ANA [8], and RF was suggested to be an independent risk factor for ischemic heart disease in men [9].

Although cases and controls were included in the study if they had no known history of an autoimmune disorder, to ensure the absence of an undiagnosed autoimmune process that could influence the results, serum was checked for anti-smooth muscle, anti-neutrophil cytoplasmic, and anti-mitochondrial antibodies. One case and 3 controls had positive anti-smooth muscle antibodies and were excluded from the statistical analysis.

**Statistical Analysis**

Data was expressed as number of cases and percentages, and measurements were presented as means ± standard deviations. Calculations were performed using the SPSS 15.0 software for Windows. Statistical significance was set at p < 0.05.

**Results**

Clinical criteria of the 57 cases and 38 controls analyzed are shown in table 1. Metabolic syndrome as a risk factor for peripheral vascular disease was significantly more prominent in patients (98.2%) compared to controls (60.5%, p < 0.05). Mean CRP, as an inflammatory marker, which is also considered as an independent risk factor for atherosclerosis, was significantly higher in patients than controls at 4.78 ± 7.70 and 2.65 ± 3.86 mg/dl, respectively (p = 0.021).

ANA were detected at a titer of ≥1:40 in 6 cases (10.5%), but none in controls. This difference, although interesting, did not reach statistical significance (p = 0.078). Noteworthy is the fact that all the cases with positive ANA had metabolic syndrome and high CRP levels.

As for RF, 4 out of 57 (7%) patients compared to 9 out of 38 (23.7%) controls had positive results (p = 0.021). Of

### Table 1. Characteristics of the study groups

|                      | Cases   | Controls | p value |
|----------------------|---------|----------|---------|
| Subjects             | 57      | 38       |         |
| Mean age ± SD, years | 60.51 ± 10.10 | 58.74 ± 10.97 | 0.420  |
| Gender, M/F          | 40/17   | 23/15    | 0.330   |
| Smokers              | 35 (61.4%) | 18 (47.4%) | 0.128   |
| Metabolic syndrome   | 56 (98.2%) | 23 (60.5%)  | 0.000   |
| Mean CRP ± SD, mg/dl | 4.78 ± 7.70 | 2.65 ± 3.86  | 0.021   |
| ANA                  | 6 (10.5%) | 0 (0%)    | 0.078   |
| RF                   | 4 (7.0%) | 9 (23.7%) | 0.021   |
| Males                | 2 (5%)  | 7 (30.4%) |         |
| Females              | 2 (11.8%) | 2 (13.3%)  |         |

RF = Rheumatoid factor.

Subjects had either disabling claudication or critical leg ischemia. Disabling claudication referred not only to claudication distance but interference with everyday living requirements and preferences. Critical limb ischemia is the term used to delineate those patients whose arterial disease has resulted in a breakdown of the skin (ulcer or gangrene) or pain in the foot even at rest; it therefore coincides with the Rutherford 4, 5, and 6 categories of the new SVS-ISCVS recommendation of reporting standards [16]. All control subjects had no clinical history of peripheral, coronary or cerebral arterial disease.
these 9 controls, 7 were males (30.4% of the total control male population); 8 exhibited features of metabolic syndrome and 7 had elevated CRP.

**Discussion**

The significance of autoantibodies in the development of atherosclerosis has been quite controversial and contrasting reports continue to accumulate. For example, while some found that anti-cardiolipin antibodies are raised in patients with coronary artery disease or that they could even represent an independent risk factor for myocardial infarction and cardiac death in middle-aged men, others could not elicit an association between anti-cardiolipin antibodies and ischemic heart disease in either men or women [9, 18, 19]. Anti-neutrophil cytoplasmic antibodies, which supposedly amplify the inflammatory process, appear to play no major role in premature atherosclerosis [20].

Similarly, contrasting data have been accumulated regarding the role of ANA in atherosclerosis. Their contribution was suggested initially from observing an accelerated atherosclerotic state in patients with systemic lupus erythematosus where ANA represent the hallmark of the disease [11–14]. While a highly predictive role of ANA in patients with coronary artery disease was suggested by Grainger and Bethell [8], who described elevated ANA in 70% of their patients with severe coronary artery disease, no similar correlation was found by other groups [9, 21]. In the present study, 10.5% of the patients with advanced PAD had positive ANA in their sera in contrast to controls. This positivity of ANA in the peripheral atherosclerosis patient group is far lower than that described by Grainger and Bethell [8] in the coronary artery disease group. Furthermore, the difference did not reach statistical significance, probably due to small sample size and genetic factors. In this study, the sample size is small. This might explain the low prevalence seen, and a future study with a larger number of cases may lead to a different conclusion. Another consideration is the genetic difference between our patient population and the population studied by Grainger and Bethell [8]. It is also plausible that the pathogenesis of peripheral arterial atherosclerosis differs significantly from that of coronary atherosclerosis. It is reasonable to conclude that positive ANA is not significantly associated with peripheral atherosclerosis in Kuwait.

The interest in RF and atherosclerosis also arose from the strong existing relationship between rheumatoid arthritis and accelerated cardiac and cerebral atherogenic processes [10, 11, 14, 15]. A role of RF per se as a pathogen was suggested by Edwards et al. [9], who reported its probable involvement in the development of ischemic heart disease in the general population, particularly men. Our study, on the other hand, showed a completely opposite result regarding male gender. Although females did not differ, RF seemed to have a negative predictive value in males with leg ischemia. The relatively higher prevalence of RF in the control group was unexpected and in the context of the original hypothesis even harder to explain.

An even more controversial issue, namely, the interaction between CRP, atherosclerosis, and autoimmunity, needs to be considered. Evidence is compelling that CRP is not simply a bystander but an essential mediator of atherosclerosis, and its plasma level correlates well with the degree of atherosclerosis [22, 23]. Interestingly, it also has the ability to bind autoantigens and presumably the capability of promoting clearance of apoptotic cells, which makes it nature’s own immunosuppressant that could protect against autoimmune disease [24–26]. Understanding how the two tasks interact may improve our future ability to interpret results related to autoantibodies and atherosclerosis.

In this study, only ANA and RF were measured. Other antibodies which might also be relevant to atherosclerosis, such as those against cardiolipin and vascular components, should be considered in further studies regarding the interaction of CRP, autoimmunity and atherosclerosis.

**Conclusion**

The incidence of the two antibodies ANA and RF in patients with peripheral arterial occlusive disease in Kuwait indicates that these two antibodies are not associated with the development of peripheral atherosclerosis. Future research is definitely required, to link autoimmunity with PAD.

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