Cardiovascular risk parameters in men with ankylosing spondylitis in comparison with non-inflammatory control subjects: relevance of systemic inflammation

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ABSTRACT

Men with AS (ankylosing spondylitis) are at elevated risk for CHD (coronary heart disease) but information on risk factors is sparse. We compared a range of conventional and novel risk factors in men with AS in comparison with healthy controls and, in particular, determined the influence of systemic inflammation. Twenty-seven men with confirmed AS and 19 controls matched for age were recruited. None of the men was taking lipid-lowering therapy. Risk factors inclusive of plasma lipids, IL-6 (interleukin-6), CRP (C-reactive protein), vWF (von Willebrand factor), fibrin D-dimer, ICAM-1 (intercellular cell-adhesion molecule-1) and fibrinogen were measured, and blood pressure and BMI (body mass index) were determined by standard techniques. A high proportion (70%) of men with AS were smokers compared with 37% of controls (P = 0.024). The AS patients also had a higher BMI. In analyses adjusted for BMI and smoking, men with AS had significantly higher IL-6 and CRP (approx. 9- and 6-fold elevated respectively; P < 0.001), fibrinogen (P = 0.013) and vWF (P = 0.008). Total cholesterol and HDL-C (high-density lipoprotein cholesterol) were lower (P < 0.05 and P = 0.073 respectively) in AS and thus the ratio was not different. Pulse pressure was also significantly higher in AS (P = 0.007). Notably, adjustment for IL-6 and CRP levels rendered all case-control risk factor differences, except pulse pressure, non-significant. In accordance with this finding, IL-6 correlated positively (r = 0.74, P < 0.001) with fibrinogen, but negatively (r = −0.46, P = 0.016) with total cholesterol concentration. In conclusion, men with AS have perturbances in several CHD risk factors, which appear to be driven principally by systemic inflammatory mediators. Inflammation-driven atherogenesis potentially contributes to the excess CHD risk in AS.

INTRODUCTION

There is substantial interest in the concept of inflammatory-driven atherogenesis in the general cardiovascular arena and, more specifically, in the autoimmune field, perhaps best exemplified by work in RA (rheumatoid arthritis). For example, work from our group [1] and others [2,3] has proposed that systemic inflammatory...
levels in RA are sufficiently elevated to influence and perturb lipids (inclusive of ‘misleadingly’ lower total cholesterol levels) and insulin action leading to greater resistance, endothelial function, and clotting and haemostatic pathways. As a result, markers of inflammation [e.g. CRP (C-reactive protein) or IL-6 (interleukin-6)], rather than conventional CHD (coronary heart disease) risk parameters, may better predict risk for vascular events in autoimmune diseases. In addition, dampening the systemic inflammatory response may be critical to attenuating vascular risk in such patients, and evidence for this proposition is emerging (reviewed in [1]).

AS (ankylosing spondylitis) is the most common form of the spondyloarthropathies, which are a cluster of chronic inflammatory rheumatic diseases with certain overlapping features: AS, Reiter’s syndrome, reactive arthritis, psoriatic arthritis, inflammatory-bowel-disease-associated spondyloarthropathy, juvenile spondyloarthropathy and undifferentiated spondyloarthropathy. The worldwide prevalence of AS is approx. 0.9 %. The pathogenesis of AS, although poorly understood, seems to be immune mediated; subchondral bone in certain joints especially sacroiliac and intervertebral joints becomes granulomatous and infiltrated with plasma cells, T-cells, macrophages and chondrocytes, with an increased release of pro-inflammatory cytokines [4].

Patients with AS are known to have an overall mortality of approx. 1.6–1.9 times that of the general population [5], and excess mortality from circulatory disease has been quoted at 20–40 % [5,6]. Despite the above observations, published data on CHD risk factor concentrations in AS are surprisingly sparse. We therefore set out to establish the pattern of conventional (lipids and blood pressure) and novel (inflammatory, haemostatic and endothelial parameters) risk factors for CHD in men with AS in comparison with healthy male controls. Risk factors examined have generally been shown to predict CHD events and most are influenced by the degree of systemic inflammation, as noted in other relevant conditions such as RA [1,7]. Our hypothesis was that, as in RA, several conventional and novel risk factors would be potentially deranged in men with AS in line with systemic markers of inflammation.

METHODS

We recruited 27 men with AS consecutively attending the seronegative spondyloarthropathies outpatient clinic in the Centre for Rheumatic Diseases at the Glasgow Royal Infirmary. To minimize confounding by sex, only male patients were recruited. Men with diabetes were excluded and, additionally, we excluded patients on lipid-lowering therapies, since the statin class of drugs may have wide-ranging effects on several risk factor pathways inclusive of systemic cytokine and CRP concentrations [1]. Male control subjects with a similar age range to the patients were recruited from local hospital staff. All patients and controls gave written informed consent, which included permission to review their medical records. The study was approved by the local Research Ethics Committee at Glasgow Royal Infirmary.

The patient’s weight and height was recorded and BMI (body mass index) was calculated. Blood pressure was recorded by the same operator using a standard mercury sphygmomanometer and appropriately sized cuff, and diastolic pressure was recorded as Korotkoff phase V. We also noted current medications and whether the subjects were current smokers. All men with AS also completed the BASDAI (Bath AS Disease Activity Index) questionnaire [8] to assess their current disease activity.

Thereafter blood for risk factor measurements [lipid profile, IL-6, CRP, fibrinogen, D-dimers and vWF (von Willebrand Factor)] was withdrawn and kept on ice until later centrifugation at 1500 g for 10 min at 4 °C. Once all the patients and controls were recruited, batch analyses were performed (to minimize inter-assay variability) for all blood parameters. It is important to note that, although we did not employ fasting, only triacylglycerol (triglyceride) concentrations were appreciably altered with non-fasting, whereas total cholesterol and HDL-C (high-density lipoprotein cholesterol) concentrations, in particular, were near identical in fasting or post-prandial samples.

Cholesterol, HDL-C and triacylglycerol were measured using commercially available enzymatic assay kits from Roche Diagnostics Corporation (Indianapolis, IN, U.S.A.) and a Hitachi 917 analyser, and LDL-C (low-density lipoprotein cholesterol) was calculated from the Friedewald equation. Fibrinogen was determined by the Clauss assay, and fibrin D-dimer (Biopool, Urea, Sweden), vWF antigen (Dako, Cambridge, U.K.), ICAM-1 (intercellular cell-adhesion molecule-1) and high sensitivity IL-6 (R & D Systems, Abingdon, Oxfordshire, U.K.) were measured by ELISA. CRP was measured using a sensitive double-antibody sandwich ELISA with rabbit anti-human CRP and peroxidase-conjugated rabbit anti-human CRP. The assay was linear up to 5 mg/l and logarithmic thereafter. The inter- and intra-assay coefficients of variation were <10 % across the range of measured results. Intra-assay coefficients of variation were <7 % for all analytes.

Statistics

Data are presented as means (S.D.). Where the data were not normally distributed, log transformations were used and geometric means (S.D.) presented. Two sample Student t tests were used initially to compare crude case-control differences. Subsequently, linear regression analysis was employed to adjust for BMI and smoking status, since the case-control difference between these
parameters was significant. Finally, we also assessed the impact of IL-6 and CRP on case-control differences in risk factor measurements by adjusting further for these parameters. Pearson’s correlations were used to examine links between IL-6, CRP levels and other risk parameters. We decided to initially adjust for IL-6 alone, since IL-6 is ‘upstream’ of the latter and responsible for its hepatic synthesis [9]. Formal power calculations were not employed due to absence of data on the majority of risk factors examined. However, our study was nearly double the size of the only other relevant study [10] in the literature which reported lower HDL-C concentrations in AS men compared with controls (n = 11 compared with 15 respectively).

RESULTS

In crude analyses (Table 1), AS patients had higher IL-6 and CRP concentrations (approx. 9- and 6-fold elevated respectively; P < 0.001), fibrinogen (P = 0.001) and vWF (P = 0.008), whereas HDL-C was significantly (P = 0.036) lower. Diastolic blood pressure and pulse pressure were also significantly lower in AS patients. However, since a high proportion (70%) of men with AS were smokers compared with the control rate of 37% (P = 0.024) and they had a mean BMI more than 2 kg/m² higher, subsequent case-control risk factor comparisons were adjusted for BMI and smoking.

Following adjustment for BMI and smoking (model A), both total cholesterol and LDL-C were significantly (P < 0.05) lower, whereas HDL-C now demonstrated a trend (P = 0.073) towards being lower in men with AS (Table 1). As a result, the cholesterol/HDL-C ratio was similar in patients and controls (4.86 compared with 4.42; P = 0.55). Otherwise, CRP, fibrinogen, vWF and diastolic blood and pulse pressures remained significantly (P < 0.05) different and in the same direction of unadjusted differences.

Further adjustment for IL-6 levels (model B) rendered all case-control risk parameter differences non-significant, except for pulse pressure (P = 0.024). In accordance with this finding, log IL-6 correlated positively with CRP and fibrinogen (r = 0.74, P < 0.001), but negatively (r = −0.46, P = 0.016) with total cholesterol concentration in men with AS (Figure 1). CRP also correlated inversely and significantly with total cholesterol and LDL-C (r = −0.48, P = 0.12; and r = −0.44, P = 0.021 respectively). We also examined case-control differences with adjustment for both IL-6 and CRP (model C).
this case, the pattern of differences was identical with the results following adjustment for IL-6 alone (Table 1). Finally, BASDAI did not correlate significantly with IL-6 or CRP levels, or with other risk factors in our cohort of AS men.

**DISCUSSION**

The present study is novel for several reasons. It is potentially the first to comprehensively examine a range of CHD risk factors in men with AS in comparison with healthy male controls of similar age. We carefully considered potential confounders inclusive of BMI and smoking and also attempted to dissect the relevance of systemic inflammation to risk factor pattern in AS by examining the influence of IL-6 and CRP concentrations. We demonstrated severalfold elevations in circulating IL-6 in AS men, a predictor of CHD events itself in the general non-inflamed population [11–13]. CRP concentrations were also substantially higher. We also noted that circulating total cholesterol and LDL-C concentrations were lower and correlated inversely with IL-6 or CRP concentrations. HDL-C was also lower in men with AS, rendering the case-control difference in the total cholesterol/HDL-C ratio to be slightly elevated in AS patients. Moreover, men with AS demonstrated significantly higher fibrinogen (>30% higher), vWF and pulse pressure levels, independent of BMI and smoking. Finally, all case-control risk factor differences, with the exception of pulse pressure, were rendered non-significant with adjustment for circulating IL-6 and CRP concentrations, thereby suggesting a key role for the systemic inflammatory response in altering risk factor patterns in AS. In this way, the results appear to parallel findings, albeit at lower systemic inflammatory levels, of data in RA [1,3].

Several of our findings warrant further discussion. Although AS is associated with less ‘inflammation’ than RA, IL-6 and CRP levels were nevertheless severalfold higher in AS relative to ‘non-inflammatory’ subjects of similar age. This finding is significant since IL-6 at chronically elevated ‘subclinical’ concentrations is linked to insulin resistance, dyslipidaemia, endothelial dysfunction and prothrombotic changes in non-inflammatory subjects [14]. Moreover, a number of prospective studies suggest that even modest elevations in IL-6 concentration, lower even than mean levels we now report in AS, predict CHD largely independently of traditional risk factors [11,12]. These studies also suggest that IL-6 may predict events better than, and independently of, elevated CRP concentrations [11,12]. Of course, IL-6 is ‘upstream’ of CRP and is responsible for its hepatic synthesis [9]. Similarly, the link between IL-6 and elevated fibrinogen is not unexpected, since IL-6 also contributes to hepatic synthesis of fibrinogen [9]. Elevations in vWF may contribute further to a hypercoagulable state in AS, although vWF is a weak predictor of CHD [15].

As a rough guide to levels of inflammation, the geometric means for CRP and IL-6 in our AS patients were slightly less than half the concentrations reported in RA patients selected for a recent trial in our hospital [16]. There is thus an apparent gradation of rising inflammation levels from non-inflammatory controls to AS to RA and the elevated systemic levels of inflammatory cytokines in AS and RA, if present over several years, might readily accelerate atherogenesis.

The consistency of reports of lower cholesterol concentrations in acute or chronic inflammatory conditions, for example in RA [1], is noteworthy. In the present study, we observed lower concentrations of both LDL-C and HDL-C in AS. Limited data from two small studies from around two decades ago are consistent with our findings. Rossner [17] reported lower total cholesterol in AS and Joven et al. [10] noted lower HDL-C in subjects with AS, but no information on potential confounders was given in these reports and interactions with the inflammatory response were not examined.

The significant inverse association of LDL-C and total cholesterol (Figure 1) with IL-6 concentrations is consistent with the literature and suggests inflammation is causally related to a reduction in LDL-C [18]. It appears as if increased LDL catabolism, rather than a lowering of synthesis, results from systemic inflammation, but whether a reduction in LDL size (considered atherogenic) or activation of the reticuloendothelial system in AS accounts for greater receptor-independent catabolism of LDL is not entirely clear and requires further investigation. Whatever the mechanism, it is important to note that receptor-independent uptake of LDL particles enhances plaque formation and atherogenesis [18], and that LDL size is significantly smaller in RA patients compared with healthy controls (reviewed in [1]), and
by extrapolation may be smaller in AS. Studies on LDL size in AS would be informative.

Our results on blood pressure findings should be treated with caution, since blood pressure was measured at a single time point and many patients with AS were on drugs (e.g. non-steroidal anti-inflammatory drugs) with potentially blood-pressure-raising effects. Moreover, we did not assess aortic root or valve disease in patients with AS [19]. Such abnormalities are common in this condition and could contribute to greater pulse pressure. Alternatively, long-standing chronic inflammation in AS may have led to stiffer vessels; higher levels of systemic inflammation whether measured by high sensitivity CRP or IL-6 concentrations are linked both to arterial stiffness and to endothelial dysfunction [20]. Future detailed studies on vascular function in AS patients seem to be warranted to disentangle potential mechanisms for greater pulse pressure. Such studies should include direct assessment of endothelial function in AS and relate such findings to both conventional risk factors and inflammatory mediators. It is worth mentioning that, whereas direct measures of endothelial function appear to independently predict CHD events [21], circulating measures are less predictive [22]. Our data on triacylglycerol concentrations in patients and controls should also be treated with caution, in view of the non-fasting (but more physiological) samples employed. We may also have overestimated BMI in some of the men with AS due to kyphosis leading to falsely low height measurements, but such inaccuracies would have led to an overadjustment for adiposity and thus attenuated case-control differences. Clearly, future studies examining metabolic and vascular risk factors in AS should include alternative measures of obesity such as waist circumference, a validated measure of central adiposity and metabolic risk [23].

In terms of clinical implications, findings from our recent statin trial [16], which suggested statin-mediated reductions not only in lipids, but also in joint disease activity, circulating IL-6 levels and a panel of other CHD risk factors in RA, indicate that such drugs may offer similar dual benefits to men with AS. Clearly, this possibility requires direct study.

In conclusion, we suggest that men with AS have perturbations in several CHD risk factors, which are driven in turn by systemic inflammatory mediators. Although it is likely that increased systemic inflammatory markers in AS patients largely reflect underlying musculoskeletal disease, inflammation-driven atherogenesis is also potentially relevant to the excess CHD risk in AS.

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