STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study.

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	Item No	Recommendation
Title and abstract	1	(*a*) Indicate study's design in title: **"cross-sectional clinical study"**
		(*b*) Abstract: **Structured abstract provided**
Introduction		
Background/rationale	2	Scientific background: **NSAID-related inhibition of aldosterone metabolism is described**
Objectives	3	State specific objectives: **"Aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA**."
Methods		
Study design	4	Key elements of study design presented early: **"Our methods have been described in detail elsewhere." [ref #13. Crilly MA, Kumar V, Clark HJ, et al .Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors. Rheumatology. 2009;48:1606-1612.] [ref # 14. Crilly MA, Clark HJ, Kumar V, et al. Relationship between arterial stiffness and Stanford Health Assessment Questionnaire disability in rheumatoid arthritis patients without overt arterial disease. J Rheumatol. 2010;37:946-952.]** **"In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration."**
Setting	5	Setting, locations, and relevant dates: **"In the original study we recruited patients … by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen."** **"Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006)."**
Participants	6	(*a*) Eligibility criteria, sources and methods of selection of participants: **"We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks."**

Variables	7	Define outcomes, exposures, and potential confounders: **"Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ). Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension)."** Diagnostic criteria: **"Consultant rheumatologist diagnosis of rheumatoid arthritis (RA)"** **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]" -- we have previously reported on the proportion of patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) who meet "American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.[Arnett FC, et al. Arthritis Rheum 1988 Mar;31(3):315-24].** **[Text from Rheumatology. 2009;48:1606-1612] "Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% met ACR (4/7) criteria for RA. This may be attributable to a typographical error in our questionnaire (which asked about morning stiffness for 6 months rather than 6 weeks). Whilst 39% of patients reported more than one hour of morning stiffness for more than 6 months in the past, only 18% of patients reported such stiffness over the previous week. Some clinical heterogeneity may exist in our study population of patients with a clinical rheumatological diagnosis of RA, although the inclusion of 'ACR criteria' as a variable in the fully adjusted analysis made no difference to our results. ACR-criteria also 'accumulates' over time. For example, in our study the median duration of arthritis was almost 10 years: some 66% (36/55) of patients with arthritis duration greater than 10 years met ACR-criteria, compared to 48% (28/59) with a shorter duration."**
Data sources/ measurement	8*	Sources of data and details of methods of assessment (measurement): **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."** **"Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from in vitro studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition). [ref #6: Knights KM, Winner LK, Elliot DJ, et al. Glucuronidation by human liver and kidney microsomes and recombinant UDP-glucuronosyltransferases: inhibition by NSAIDs. Br J Clin Pharmacol 2009;68:402-412.] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis."**
Bias	9	Efforts to address potential sources of bias: **"BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA)." [El Assaad MA, et al. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. Blood Press Monit 2003;8:127-133.]**

		"**A detailed retrospective review of the medical records, using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results".** **"The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed".**
Study size	10	Study size: **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]"** **The original study recruited 114 patients.** **[Text from Rheumatology. 2009;48:1606-1612] "A sample size of 110 patients with RA was our intention, in order to ensure that there were 10 subjects for each CV risk factor included in the multi-variable analysis. We included 11 cardiovascular risk factors in our analysis, based on those contributing 90% of the 'population attributable risk' to myocardial infarction in the recent INTERHEART study."**
Quantitative variables	11	Quantitative variables handled: **AIX% and RWT were Normally distributed and summarised as mean (SD).** **"Analysis is based on the mean of the three PWA measurements"** Groupings chosen: **Not applicable**
Statistical methods	12	(*a*) Statistical methods (including control for confounding): **Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years.**
		(*b*) Subgroups and interactions: **Not applicable**
		(*c*) Missing data: **We had negligible levels of missing data due to the implementation of a rigorous study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Except for the calculation of 'ESR-years' (as a measure of cumulative inflammatory burden) the level of missing data was minimal.** **"Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]"** **[Text from Rheumatology. 2009;48:1606-1612] "Medical records were available for 112 patients (complete medical records could not be obtained for two patients) who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years. Missing ESR data was imputed as previously described. For individual patients the average availability of an annual ESR, for each year since the onset of arthritis, was a median of 93% (IQR 67% to 100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor, ACR-RA criteria or Stanford disability index (data not shown)."** **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**

		(*d*) Analytical methods taking account of sampling strategy: **Not applicable**
		(e) Sensitivity analyses: **"The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study: **"The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid)."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]" – we had negligible levels of missing data due to the implementation of a very careful study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Of the 114 patients recruited 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]** **We have no data available concerning the number of RA patients excluded at the rheumatology clinic level because they were already known to have arterial disease.** **We also have no data concerning the number of eligible RA patients attending rheumatology clinic who declined to be contacted by our research nurse. All of the RA patients who did agree to being contacted by the research nurse subsequently attended for assessment (N=114) and have been included in this analysis as described.** **No patients were excluded because of technical failure to undertake PWA. Despite initial (pre-study) concerns that it might not be feasible to undertake PWA in some RA patients, due to diseased wrists, this proved not to be the case. The research nurse successfully undertook PWA on all participants.**
		(b) Reasons for non-participation: **Not known/recorded**
		(c) Flow diagram: **None/ not applicable**
Descriptive data	14*	(a) Characteristics of study participants: **Shown in detail in Table 1**
		(b) Indicate number of participants with missing data: **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**
Outcome data	15*	Report summary measures: **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."**
Main results	16	(*a*) Unadjusted estimates and confounder-adjusted estimates (95%CI): **Table 3 reports unadjusted and adjusted mean differences in AIX% and RWT (with their related 95%CI's)** Make clear which confounders were adjusted for and why they were included **"Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of**

		linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."
		(*b*) Category boundaries (continuous variables categorized): **Not applicable**
		(*c*) Estimates of absolute risk: **Not applicable**
Other analyses	17	Subgroup analysis: **None** Sensitivity analyses: **see STROBE #12e (above)**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **"In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac and lowest in those taking ibuprofen. These difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, since the observed differences remained after adjusting for such features. Consequently differences in AIX and RWT appear to be genuinely related to the use of different ns-NSAIDs. Patients using ns-NSAIDS with intermediate levels of AGI, naproxen and indomethacin, demonstrated intermediate levels of arterial dysfunction."**
Limitations	19	Limitations of the study, sources of potential bias or imprecision: **"The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA we are unable to directly confirm the previous in vitro findings. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. …. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings."**
Interpretation	20	Cautious overall interpretation of results: **"Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction."**
Generalisability	21	Generalisability (external validity) of the study results: **"The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK. [ref #32. Panoulas VF, Douglas KM, Milionis HJ et al. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology 2007;46:1477-1482]"**
Other information		
Funding	22	Source of funding and role of the funders: **"The original study was supported by charitable funding from NHS Grampian Rheumatology Endowments. The funders played no role in the analysis or reporting of this study."**

*Give information separately for exposed and unexposed groups.