

## Atherothrombotic events in rheumatoid arthritis are predicted by homocysteine – a six-year follow-up study

S. Berglund, A. Södergren,  
S. Wållberg Jonsson,  
S. Rantapää Dahlqvist

Department of Public Health and Clinical  
Medicine, Rheumatology, University  
Hospital, Umeå, Sweden.

Sarah Berglund, MD

Anna Södergren, MD

Solveig Wållberg Jonsson, MD

Solbritt Rantapää Dahlqvist, MD, PhD

This study was supported by grants from  
the Swedish Research Council (K2007-  
52X-20307-01-3), King Gustaf V's  
80-Year Fund, the Swedish Rheumatism  
Association, and the Medical Faculty  
of Umeå University, Umeå, Sweden.

Please address correspondence to:  
Solbritt Rantapää Dahlqvist, MD, PhD,  
Department of Public Health and Clinical  
Medicine, Rheumatology,  
University Hospital, SE-90185 Umeå,  
Sweden.

E-mail:

Solbritt.Rantapaa.Dahlqvist@medicin.umu.se

Received on December 19, 2008; accepted  
in revised form on April 14, 2009.

© Copyright CLINICAL AND  
EXPERIMENTAL RHEUMATOLOGY 2009.

**Key words:** Homocysteine,  
rheumatoid arthritis, atherothrombotic  
event, cardiovascular risk factors.

## ABSTRACT

**Objective.** The aim of this study was to investigate whether homocysteine is linked to atherothrombotic (AT) events in patients with rheumatoid arthritis (RA).

**Methods.** Analysis of homocysteine (Hcy) levels was carried out in 235 consecutive RA patients. They were followed-up for 6.5 years or until death, with analysis of AT risk factors and the type and length of DMARD and corticosteroid treatment. The disease history before inclusion was collected. Six categories of AT events were defined. In addition, the diagnosis of the patients at follow-up was co-analyzed with the nationwide population-based Swedish Inpatient Register and Death Register to certify all events.

**Results.** The Hcy level was found to be higher in males ( $p < 0.05$ ) and increased with age ( $p < 0.001$ ). Patients with folic acid supplementation had significantly lower levels, while those on corticosteroids had higher levels. High Hcy levels predicted AT events ( $n = 48$ ) during a 6.5-year follow-up adjusted for age and male sex in a logistic regression analysis.

**Conclusion.** In this study, RA patients on folic acid had lower Hcy levels. High Hcy levels (in addition to age, sex and diabetes) predicted AT event prospectively.

## Introduction

Increased levels of homocysteine (Hcy) have in retrospective studies of the general population been associated with an increased risk of atherothrombotic (AT) disease (1, 2). The findings from prospective studies evaluating increased levels of Hcy in cardiovascular disease (CVD) have been inconsistent, although case-control studies support the existence of an association (3). Previous studies of patients with rheumatoid arthritis (RA) have shown increased levels of Hcy compared with controls (4-8). This is of interest because it has been shown that individuals with RA are at increased risk of morbidity and mortality due to cardiovascular disease, which cannot be explained solely by the presence of traditional CV risk factors. Non-traditional risk factors linked to

the inflammatory burden of the disease (7, 9) and/or a genetic predisposition (10, 11) have been implicated in the accelerated atherosclerosis observed in RA (12, 13).

There are studies demonstrating that methotrexate (Mtx) promotes hyperhomocysteinemia, possibly through the depletion of folate (14, 15). Other classic disease-modifying anti-rheumatic drugs (DMARDs) have also been found to influence the Hcy level in one direction or the other (16, 17). The precise molecular mechanism of this process is still not completely understood, but it has been suggested that hyperhomocysteinemia may promote endothelial oxidative damage and dysfunction, which is an early step in the development of atherosclerosis (2, 5). The aim of this study was to investigate whether homocysteine levels could be linked to an increased incidence of CV morbidity and mortality during a 6.5-year follow-up.

## Materials and methods

During April and May 2000 all patients visiting the Department of Rheumatology of the University Hospital Umeå who fulfilled the criteria for RA (18) ( $n = 235$ ) were consecutively enrolled in a prospective 6.5-year follow-up study. A sub-cohort of those patients with Hcy levels  $\geq 12 \mu\text{mol/L}$  was included in a randomized controlled 6-month trial of treatment with B-vitamins (19).

Data was collected on all patients using a structured registration form ( $n = 235$ ); this included all hospital records retrospectively from disease onset until 2000 and prospectively from 2000 until 2006 or death. The records could not be located for one patient, so that no information was available except for the end-point data.

From disease onset until 2006 or death, pharmacologic treatment with corticosteroids and DMARDs (methotrexate, anti-malarials, cyclosporine, aurothiomalate, sulfasalazine, auranofine, leflunomide, azathioprine, tumour necrosis factor antagonists, anti-CD20 antibody or d-penicillamine) was recorded. In our patient cohort 97% had been prescribed DMARDs at some time during the disease course and 76% had taken corticosteroids.

Competing interests: none declared.

**Table I.** Clinical data on the RA patients, stratified for sex at the time of sampling for homocysteine in 2000.

	Male (n=68)	Female (n=166)
Age, mean ( $\pm$ SD), years	52 $\pm$ (16)**	46 $\pm$ (16)
RA duration, mean ( $\pm$ SD), years	11.7 $\pm$ (9.0)*	15.2 $\pm$ (12.4)
Hcy, mean ( $\pm$ SD), $\mu$ mol/L	12.8 $\pm$ (4.5)*	11.4 $\pm$ (4.0)
ESR, mean ( $\pm$ SD), mm/h	25 $\pm$ (19.1)	27 $\pm$ (20.3)
Extra-articular disease	14 (26.5%)	38 (22.9%)
DMARDs	48 (71%)	128 (77%)
Methotrexate	22 (32%)	80 (48%)*
Corticosteroids, oral (<10 mg/day)	30 (45%)	17 (47%)
Folate substitution	17 (25.4%)	57 (34.8%)
Hypertension	28 (41%)	73 (44%)
Hyperlipidemia	1 (1.5%)	9 (5.4%)
Diabetes mellitus	12 (18%)	16 (10%)
Smoker ever	31 (46%)	57 (34%)

\* $p < 0.05$ , \*\* $p < 0.01$ .

The presence of RA complications during disease progression – *i.e.* vasculitis, neuropathy, pleuritis, pericarditis, rheumatoid nodules or scleritis – was documented. Established CV risk factors – *i.e.* hypertension (with treatment or no treatment), hyperlipidemia (diagnosed as hypercholesterolemia and/or hypertriglyceridemia), diabetes mellitus (insulin or tablet treatment) and smoking (ever or never) – were evaluated and registered at inclusion. Fatal or non-fatal atherothrombotic events (AT) before 2000 were recorded as previous events. AT events during follow-up were identified and placed in one of six categories: myocardial infarction (MI) was diagnosed according to the Minnesota code (20); ischaemic heart disease (IHD) was verified by coronary artery bypass graft surgery or percutaneous coronary intervention; stroke was registered when intra-cerebral haemorrhage or infarction had been diagnosed following computerized tomography or magnetic resonance imaging, or when a typical clinical picture with neurological deficits had persisted for more than 24h; transient ischaemic attack (TIA) was registered in cases where focal neurological deficit of presumed ischaemic origin had persisted for less than 24 h; deep vein thrombosis/pulmonary embolism (DVT/PE) was recorded when the diagnosis had been verified by objective tests (*e.g.* phlebography, sonography, scintigraphy, and/or arteriography) or when clinical

signs combined with pulmonary radiography, electrocardiography, and laboratory changes led to full-time treatment with warfarin. Peripheral embolism was diagnosed by angiography or when acute amputation was necessary, aortic aneurysm was diagnosed in the presence of standard symptoms. In the case of fatal CV events, information was obtained from death certificates. Furthermore, each patient was linked through his or her unique identification number to the Swedish Cause of Death Register (date of death) and the population-based Swedish Inpatient Register (which contains information on all inpatient care, including dates and medical discharge diagnoses [coded according to the International Classification of Diseases, version 10]) in order to verify the diagnosis from the follow-up.

Serum was collected at inclusion, centrifuged within 30–60 minutes, and homocysteine ( $\mu$ mol/L) was measured by fluorescence polarization immunoassay (IMX system, Abbot, Oslo, Norway) as previously described (17). At the same time the erythrocyte sedimentation rate (ESR) (mm/h) was analyzed. Current treatment, the presence of traditional risk factors, and previous TE events registered at inclusion are presented in Table I.

The Regional Ethics Committee at the University Hospital, Umeå, approved this study and all participants gave their written informed consent.

### Statistical analysis

Chi-square tests were used to compare differences between the categorical data; continuous data were compared using Students' *t*-test for independent samples. Correlations were analyzed by Pearson's product moment correlation (*r*). To identify predictors for CV events during follow-up, Kaplan-Meier and Cox proportional hazard regression analyses were used. The Hcy level was stratified into two groups, high ( $n=106$ ) and low ( $n=126$ ), defined as more or less than the median value ( $>11 \mu$ mol/L and  $\leq 11 \mu$ mol/L, respectively). Calculations were performed using SPSS 14.0 for Windows (SPSS for Mac 14.0: SPSS Inc. Chicago, IL, USA).

### Results

At sampling the Hcy level was significantly increased in males –  $12.8 \pm 4.5 \mu$ mol/L versus  $11.4 \pm 4.0 \mu$ mol/L in females ( $p < 0.05$ ) – and the levels were significantly correlated ( $p < 0.001$ ) with age in both cases (males:  $r=0.349$ ,  $p < 0.01$  and females:  $r=0.306$ ,  $p < 0.001$ , respectively). Besides sex, increasing age ( $\beta=0.099$ , 95%CI 0.062–0.136,  $p < 0.001$ ) and the presence of hypertension ( $\beta=1.11$ , 95%CI (–0.06)–2.27,  $p=0.063$ ) predicted increased levels of Hcy. The presence of diabetes, extra-articular disease, ever smoking, AT events before 2000, hyperlipidemia and disease duration were not correlated with Hcy levels. Patients with folic acid substitution at the time of sampling had significantly lower Hcy levels, *i.e.*,  $10.6 \pm 0.5 \mu$ mol/L versus  $12.6 \pm 0.3 \mu$ mol/L in those without folic acid substitution ( $p < 0.01$ ), irrespective of DMARD treatment. Patients on oral corticosteroids at sampling had significantly higher Hcy levels ( $12.4 \pm 0.4 \mu$ mol/L versus  $11.3 \pm 0.4 \mu$ mol/L,  $p < 0.05$ ) and higher ESR ( $31.1 \pm 2.2$  mm/h versus  $22.6 \pm 1.5$  mm/h,  $p < 0.001$ ) compared to those not on corticosteroids. Only the lack of folic acid supplementation ( $p < 0.05$ ) and age remained significant predictors for increased Hcy levels in a multiple regression analysis that took into account the above-mentioned factors.

The follow-up period for this study was 6.5 years, during which time 38 patients died. In the follow-up period 48

patients suffered one or more events: 28 AMI (8 fatal), 14 stroke/TIA, 6 DVT/PE (2 fatal), and 2 peripheral embolism/aorta aneurysm. Fourteen patients had undergone coronary bypass surgery/PCI in connection with their AMI. After disease onset and up to the year 2000 there were 41 AT events: 18 patients with AMI, 17 cases with stroke/TIA, 9 with DVT/PE, 5 with CABG, and 1 with peripheral embolism. These events were evenly distributed between the sexes. Three events occurred before the onset of RA. Men had a 5-fold higher frequency (13/68) of more than one AT event compared to women (6/167).

AT events during follow-up were predicted by sex and by a high Hcy level ( $p<0.001$ ), the presence of DM ( $p<0.001$ ) and hypertension ( $p<0.01$ ), and an AT event before inclusion (year 2000) ( $p<0.001$ ), when analyzed by Kaplan-Meier log rank tests. Based on Cox proportional hazard regression analysis (Table II), high Hcy levels adjusted for age and sex were a significant predictor of AT events. When hypertension was included in the model the predictive value was reduced; for Hcy  $\beta=1.84$ , CI95% 0.92–3.69,  $p=0.084$ , and for hypertension  $\beta=1.71$ , CI95% 0.95–3.07,  $p=0.073$ . The predictive value of Hcy was further reduced when DM was added to the original model (Table II), while DM alone, adjusted for age and sex, predicted AT events significantly ( $\beta=3.14$ , CI95% 1.44–6.84,  $p=0.004$ ). Adjustments for folic acid or B-vitamin supplements during the treatment trial did not change the predictive value of the factors under consideration. Smoking (defined as smoking ever) was not significantly related to AT events. The relatively low number of each type of event did not allow us to conduct separate analyses of the predictors for these events.

## Discussion

In the present study, high levels of Hcy predicted AT events in RA patients during the time frame of a 6.5-year follow-up. The relationship between high Hcy levels and an AT event remained significant after adjustment for sex and age. Hypertension did not remain a significant predictor after such adjustments.

**Table II.** Two separate models of Cox proportional hazard regression analysis in 235 RA patients, with CV events during follow-up as the dependent variable.

	$\beta$	95% CI	$p$ -value	$\beta$	95%CI	$p$ -value
Sex, male/female	2.22	1.23 – 4.02	<0.01	1.84	0.99 – 3.40	0.05
Age	1.06	1.03 – 1.09	<0.001	1.07	1.04 – 1.10	<0.001
HCY, high/low	1.96	0.99 – 3.90	0.05	1.80	0.90 – 3.59	0.096
DM	–	–	–	3.55	1.69 – 7.46	<0.001

Our results suggest that Hcy should be regarded as a risk factor of equivalent significance as certain other factors. Previous studies have shown a relationship between hyperhomocysteinemia and CVD and AT in the general population (1–3). In patients with systemic lupus erythematosus, increased levels of Hcy predicted stroke and arterial disease, but not venous thrombotic disease (21). However, to the best of our knowledge this represents the first study of RA patients in which a relationship between higher Hcy levels and future AT events has been demonstrated. Our results are supported by the finding of significantly increased Hcy levels in RA patients positive for antiphospholipid antibodies (22). In that retrospective study RA patients with thromboses ( $n=20$ ) had significantly higher levels of Hcy (22).

It has been proposed that elevated Hcy levels in patients with RA may be the result of Mtx treatment (14, 15). On the contrary, the patients in our study who were treated with Mtx presented low Hcy levels. However, there is a strong indication that this could be due to folic acid supplementation, which was related to the lowest level of Hcy. We also found that patients on oral corticosteroids had higher levels of Hcy, as well as a higher ESR. We concluded from this finding that patients with the highest activity were on corticosteroids, which could explain the increased Hcy levels in these patients. A bi-directional relationship between Hcy and inflammation has been suggested (23). The Hcy concentration has been reported to be positively related to inflammatory markers, and enhanced cytokine production in RA synoviocytes was shown to be induced by Hcy. Although we did not find a correlation between ESR and Hcy levels in this follow-up study, we did detect a positive relation-

ship between reductions in Hcy levels and CRP levels in the RCT (19). This finding has been confirmed in a recent study, where a significant association was reported between increased Hcy and CRP, and between Hcy and radiologic damage (24).

DMARD treatment has been linked to a reduced frequency of CVD in RA; however, in this study a separate effect on the Hcy level was not found with DMARD treatment at sampling, adjusted for folic acid supplementation. Furthermore, vitamin treatment trials with homocysteine-lowering supplements have failed to demonstrate a reduced risk of CVD in the general population (17, 25). In agreement with others, we found that men have increased levels of Hcy compared with women (2, 17). It has been suggested that estrogens could decrease Hcy levels and that this is the reason why pre-menopausal women exhibit lower Hcy levels than both men and post-menopausal women (17). In this study, the Hcy level was related with age in both sexes, which is consistent with previous reports (17). As in earlier studies, the baseline levels of Hcy among RA patients was comparatively high,  $12\pm4.2$   $\mu\text{mol/L}$ .

The main limitation of our study is that the patients were not followed prospectively from disease onset, but retrospectively from inclusion and prospectively only from the date of inclusion for a period of 6.5 years. The data recorded at the time of sampling on disease activity was limited. It would have been of interest if more inflammatory markers and the joint count had been assessed. The only marker of disease activity that could be used in this study was the ESR. In addition, factors other than drug treatment that might have influenced the Hcy level, including metabolic diseases, gastrointestinal and renal dysfunctions, and various

deficiencies (2, 17), could not be considered in detail in this study.

In conclusion, high Hcy levels, in addition to age, sex and diabetes, are predictive of AT events in RA patients.

## References

1. WIERZBICKI AS: Homocysteine and cardiovascular disease: a review of the evidence. *Diab Vasc Dis Res* 2007; 4: 143-50.
2. ELDIBANY MM, CAPRINI JA: Hyperhomocysteinemia and thrombosis: an overview. *Arch Pathol Lab Med* 2007; 131: 872-84.
3. FORD ES, SMITH SJ, STROUP DF, STEINBERG KK, MUELLER PW, THACKER SB: Homocyst(e)ine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. *Int J Epidemiol* 2002; 31: 59-70 [review].
4. PETTERSSON T, FRIMAN C, ABRAHAMSSON L, NILSSON B, NORBERG B: Serum homocysteine and methylmalonic acid in patients with rheumatoid arthritis and cobalaminopenia. *J Rheumatol* 1998; 25: 859-63.
5. HERNANZ A, PLAZA A, MARTÍN-MOLA E, DE MIGUEL E: Increased plasma levels of homocysteine and other thiol compounds in rheumatoid arthritis women. *Clin Biochem* 1999; 32: 65-70.
6. WÄLLBERG JONSSON S, CVETKOVIC JT, SUNDQVIST KG, LEFVERT AK, RANTAPÄÄ-DAHLQVIST S: Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2002; 29: 875-82.
7. CISTERNAS M, GUTIÉRREZ MA, KLAASSEN J, ACOSTA AM, JACOBELLI S: Cardiovascular risk factors in Chilean patients with rheumatoid arthritis. *J Rheumatol* 2002; 29: 1619-22.
8. ROUBENOFF R, DELLARIPA P, NADEAU MR *et al.*: Abnormal homocysteine metabolism in rheumatoid arthritis. *Arthritis Rheum* 1997; 40: 718-22.
9. WÄLLBERG-JONSSON S, JOHANSSON H, ÖHMAN M-L, RANTAPÄÄ DAHLQVIST S: Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. *J Rheumatol* 1999; 26: 2562-71.
10. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, LOPEZ-DIAZ MJ *et al.*: HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. *Arthritis Care Res* 2007; 57: 125-32.
11. ARLESTIG L, WÄLLBERG JONSSON S, STEGMAYR B, RANTAPÄÄ-DAHLQVIST S: Polymorphism of genes related to cardiovascular disease in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2007; 25: 866-71.
12. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005; 35: 8-17.
13. DESSEIN PH, JOFFE BI, VELLER MG *et al.*: Traditional and non-traditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2005; 32: 435-42.
14. MORGAN SL, BAGGOTT JE, LEE JY, ALARCÓN GS: Folic acid supplementation prevents deficient blood folate levels and hyperhomocysteinemia during long-term, low-dose methotrexate therapy for rheumatoid arthritis: implications for cardiovascular disease prevention. *J Rheumatol* 1998; 25: 441-6.
15. SLOT O: Changes in plasma homocysteine in arthritis patients starting treatment with low-dose methotrexate subsequently supplemented with folic acid. *Scand J Rheumatol* 2001; 30: 305-7.
16. HAAGSMA CJ, BLOM HJ, VAN RIEL *et al.*: Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. *Ann Rheum Dis* 1999; 58: 79-84.
17. REFSUM H, AMITH AD, UELAND PM *et al.*: Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 2004; 50: 3-32.
18. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
19. ARLESTIG L, WÄLLBERG JONSSON S, STEGMAYR B, RANTAPÄÄ-DAHLQVIST S: Homocysteine in patients with rheumatoid arthritis in relation to inflammation and B-vitamin treatment. *Scand J Rheumatol* 2003; 32: 205-10.
20. PRINEAS RJ, CROW RJ, BLACKBURN H: *The Minnesota Code Manual of Electrocardiographic Findings: Standards and Procedures for Measurement and Classification*. Boston, MA, John Wright & Son Ltd., 1982.
21. PETRI M, ROUBENOFF R, DALLAL GE, NADEAU MR, SELHUB J, ROSENBERG IH: Homocysteine is a potentially modifiable, independent risk factor for stroke and thrombotic events in patients with SLE. *Lancet* 1996; 348: 1120-4.
22. SERIOLO B, FASCILOLO D, SULLI A, CUTOLO M: Homocysteine and antiphospholipid antibodies in rheumatoid arthritis patients: relationships with thrombotic events. *Clin Exp Rheumatol* 2001; 19: 561-4.
23. LAZZERINI PE, CAPECCHI PL, SELVI E *et al.*: Hyperhomocysteinemia, inflammation and autoimmunity. *Autoimmun Rev* 2007; 6: 503-9.
24. LOPEZ-OLIVO MA, GONZALEZ-LOPEZ L, GARCIA-GONZALEZ A *et al.*: Factors associated with hyperhomocysteinemia in Mexican patients with rheumatoid arthritis. *Scand J Rheumatol* 2006; 35: 112-6.
25. BØNAA KH, NJOLSTAD I, UELAND PM *et al.*: Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006; 354: 1578-88.