

# Serum copper: a marker of disease activity in rheumatoid arthritis

AAR YOUSSEF, B WOOD\*, DN BARON

*From the Royal Free Hospital and School of Medicine, London and \*Whittington Hospital, London*

**SUMMARY** Serum copper concentrations were measured in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS), osteoarthritis (OA), and in healthy controls. Median serum copper concentrations were raised significantly in RA and AS, but not in OA. Serum copper in RA correlated significantly with a number of disease activity markers—for example erythrocyte sedimentation rate (ESR), C-reactive protein, haemoglobin concentration, morning stiffness, and grip strength. It also correlated well with the overall disease activity as assessed by a composite index. Raised serum copper was associated with severe RA as manifested by the presence of immunoglobulin M rheumatoid factor, extra-articular features, weak grip and highly active disease. High serum copper might be related to the development of the pathological lesions observed in RA and not just be a secondary response.

The finding of abnormally high serum/plasma copper concentration in patients with rheumatoid arthritis (RA) has attracted interest.<sup>1-5</sup> This interest has been further stimulated by the discovery that copper complexes of anti-inflammatory agents are more potent than the parent compounds.<sup>6,7</sup> Furthermore, it has been claimed (though not generally accepted) that the traditional use of copper bracelets by arthritic patients results in improvement.<sup>8,9</sup>

On the other hand, many workers,<sup>10-12</sup> but not all,<sup>13</sup> have shown a correlation of serum copper concentration with disease activity in RA.

The purpose of the present study was to cast further light on the significance of raised serum copper in RA patients, particularly its relation to disease activity. The latter was assessed by a newly developed composite activity index (CAI).<sup>14,15</sup> Serum copper was also measured in healthy controls and in patients with either osteoarthritis (OA) or ankylosing spondylitis (AS).

## Patients and methods

### PATIENTS

The following were included: 60 patients with classical or definite RA as defined by the criteria of the American Rheumatism Association;<sup>16</sup> 19 patients with OA diagnosed clinically and radiologically; 10 patients with AS diagnosed by the New York criteria;<sup>17</sup> and 14 apparently healthy subjects used as controls. The composition of the groups is shown in Table 1. Serological tests for immunoglobulin M rheumatoid factor (IgMRF) were positive in 41 RA patients. RA patients receiving

oestrogen-containing oral contraceptives (known to increase serum copper values<sup>18,19</sup>) or wearing copper bracelets were excluded. All patients were receiving different forms of antirheumatic therapy.

Assessment of the overall disease activity in the RA patients was performed using a CAI, including both clinical and laboratory markers (Table 2) which were combined to form a single score.<sup>14,15</sup> Using the CAI score the 60 RA patients were classified into slightly, moderately, and highly active.

### CLINICAL INVESTIGATIONS

The following were measured according to procedures described in detail elsewhere:<sup>14,15</sup> duration of morning

Table 1 *Composition of patient and control groups*

Group	Sex		Age (yr)	
	Male	Female	Median	Range
RA	20	40	63	37-84
OA	2	17	62	40-81
AS	9	1	52.5	32-76
Controls	5	9	58	33-76

Table 2 *Relation between serum copper concentration ( $\mu\text{mol/l}$ ) and each CAI criterion*

Composite activity index criteria	No of patients	Correlation coefficient	<i>p</i>
CRP	60	<i>r</i> = 0.51	<0.001
ESR	60	<i>r</i> = 0.47	<0.001
Hb	58	<i>r</i> = -0.43	<0.001
MS	60	<i>r</i> = 0.26	<0.05
AI	60	<i>r</i> = 0.06	NS
Joint pain	53	<i>r<sub>s</sub></i> = 0.13	NS

*r* = Pearson's correlation coefficient

*r<sub>s</sub>* = Spearman's rank correlation coefficient

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stiffness (MS) (min), joint pain, articular index (AI), grip strength (GS), the number of tender joints.

LABORATORY INVESTIGATIONS

The following were measured according to standard laboratory methods:<sup>14,15</sup> IgMRF, C-reactive protein (CRP), immunoglobulin (IgG, IgM, IgA), serum albumin, serum alkaline phosphatase (ALP) serum  $\gamma$ -glutamyltransferase (GGT), erythrocyte sedimentation rate (ESR), total white blood count (WBC), haemoglobin (Hb), platelet count.

Serum copper was measured by a modification of the method described by Piper and Higgins,<sup>20</sup> using a double beam atomic absorption spectrophotometer (Perkin-Elmer model 306, Beaconsfield, Buckinghamshire). Protein free extracts were prepared by diluting serum with one volume of 200 g/l trichloroacetic acid, followed by mixing and centrifugation. The supernatants were then aspirated directly into the air-acetylene flame.

STATISTICAL ANALYSIS

Comparison between groups was performed by Mann-Whitney U test (two tailed). Correlations were calculated using either Pearson's correlation coefficient ( $r$ ) or Spearman's rank correlation coefficient ( $r_s$ ).

Results

COMPARISON BETWEEN SERUM COPPER CONCENTRATIONS IN PATIENTS WITH RA, OA, AS, AND NORMAL CONTROLS (Fig. 1)

Patients in the RA group had a median serum copper value ( $26.0 \mu\text{mol/l}$ ) significantly higher than that of the normal control group ( $21.8 \mu\text{mol/l}$ ;  $p < 0.001$ ) and the OA group ( $21.6 \mu\text{mol/l}$ ;  $p < 0.001$ ), but not different from that of the AS group ( $26.4 \mu\text{mol/l}$ ). Mean  $\pm$  SD ( $\mu\text{mol/l}$ ): control  $21.4 \pm 3.7$ , reference range  $14.0\text{--}21.8$ ; RA  $26.9 \pm 6.7$ , OA  $20.9 \pm 2.7$ ; AS  $25.6 \pm 6.4$ .

SERUM COPPER IN PATIENTS WITH RA

The serum copper level in 60 RA patients showed no correlation with either the patient's age or the duration of disease. Median serum copper concentrations in male ( $24.0 \mu\text{mol/l}$ ) and female ( $26.5 \mu\text{mol/l}$ ) patients were not significantly different.

RA patients with IgMRF+ve had a significantly higher median serum copper concentration ( $29.5 \mu\text{mol/l}$ ) than that of patients with IgMRF-ve ( $23.4 \mu\text{mol/l}$ ;  $p < 0.03$ ).

RA patients with extra-articular manifestations had a higher median serum copper ( $29.0 \mu\text{mol/l}$ ) than that of patients without them ( $24.4 \mu\text{mol/l}$ ). However, the difference was not significant.

RA patients treated with gold, penicillamine or corticosteroids had a median serum copper ( $25.4 \mu\text{mol/l}$ ) not significantly different from that of those treated with non-steroidal anti-inflammatory drugs ( $26.2 \mu\text{mol/l}$ ).

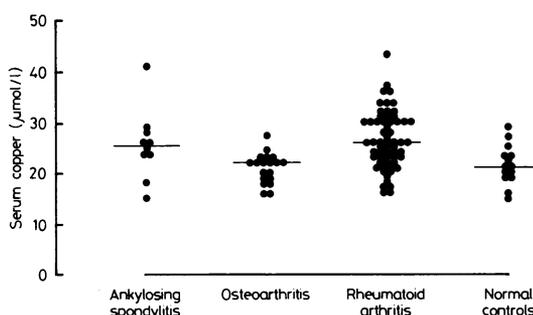


Fig. 1 Serum copper concentrations in different groups of patients and controls. Bars indicate the median.

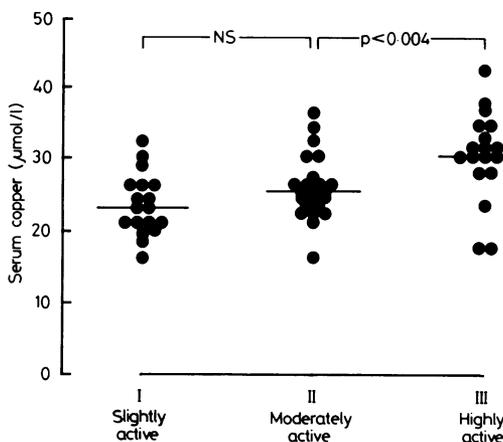


Fig. 2 Serum copper concentrations in different disease activity groups of rheumatoid arthritis. Bars indicate the median.

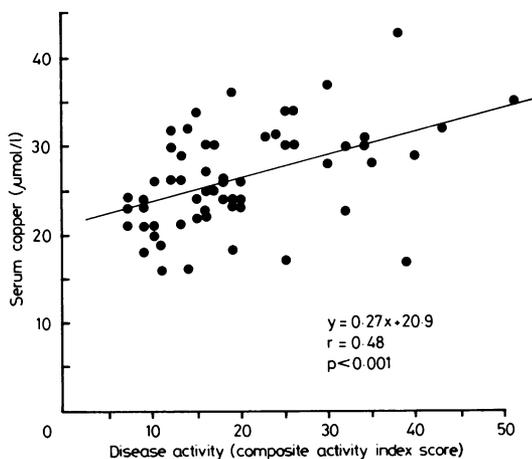


Fig. 3 The relation between serum copper concentration ( $\mu\text{mol/l}$ ) and disease activity measured with the composite activity index (CAI) in 60 patients with rheumatoid arthritis.  $r$  = Pearson's correlation coefficient.

The median serum copper concentration of 19 RA patients in the highly active group (30.5  $\mu\text{mol/l}$ ) was significantly higher than that of RA patients in both the moderately active and the slightly active groups ( $p < 0.004$  and  $p < 0.001$  respectively; Fig. 2).

Highly significant correlation was found between serum copper value and disease activity as measured by the CAI ( $r = 0.48$ ,  $p < 0.001$ ; Fig. 3).

The correlation of serum copper concentration with each individual criterion forming the CAI is given in Table 2.

The relation between serum copper concentration and other factors believed to be related to disease activity in RA is shown in Table 3.

### Discussion

Our results support the previous reports of raised serum copper both in RA<sup>1-5</sup> and in AS<sup>11, 21</sup>, not related to age, disease duration or drug administration.

Most of the copper is tightly bound to a specific carrier protein caeruloplasmin. This protein is known to behave as an acute phase reactant which increases non-specifically in response to inflammation.<sup>22</sup> Caeruloplasmin analysis was not part of the present study. There is ample evidence of its strongly positive correlation with serum copper—for example, Scudder *et al*<sup>2</sup> have reported  $r = 0.86$   $p < 0.001$  in normals and  $r = 0.84$   $p < 0.001$  in RA. This is supported by other studies in normals<sup>21, 23</sup> and in RA.<sup>3, 4</sup> RA is a chronic inflammatory disease with acute exacerbations, and an increase in serum acute phase proteins. Therefore, it is likely that in our RA patients, raised serum copper was a secondary result of high concentrations of caeruloplasmin rather than a primary disturbance of copper metabolism. This explanation is supported by our finding that the acute phase protein CRP and the ESR (which is influenced by the acute phase protein fibrinogen<sup>24</sup>) were raised in RA patients. Both CRP and ESR correlated highly significantly with serum copper values. On the other hand, albumin, which is not an acute phase protein, was not significantly altered, and showed no relation with serum copper. Further evidence

Table 3 Relation between serum copper concentration and other factors related to rheumatoid activity

Factor	No of patients	Correlation coefficient	<i>p</i>
1 - GS (mean of both hands)	58	$r = 0.45$	$<0.001$
2 - IgMRF (titre)	60	$r_s = 0.38$	$<0.004$
3 - ALP	46	$r = 0.37$	$<0.05$
4 - IgG	23	$r = 0.39$	NS
5 - IgA	23	$r = 0.36$	NS
6 - Platelet count	48	$r = 0.27$	NS
7 - Serum albumin	55	$r = 0.20$	NS
8 - IgM	23	$r = -0.18$	NS
9 - GGT	45	$r = 0.13$	NS
10 - WBC	59	$r = -0.09$	NS
11 - No of tender joints	60	$r = -0.008$	NS

$r$  = Pearson's correlation coefficient

$r_s$  = Spearman's rank correlation coefficient

that copper concentrations in RA reflect the acute phase response was obtained by our finding that a similar rise in serum copper occurs in AS, an inflammatory condition also known to be associated with an acute phase response.<sup>21</sup> Moreover, we did not demonstrate raised copper concentrations in OA, a degenerative condition not associated with an acute phase response.

Based on the above results, a significant correlation was found between serum copper and the overall disease activity as measured by the CAI which included both laboratory and clinical markers of disease activity: patients assessed as having highly active disease showed significantly higher median serum copper values than patients with either slightly or moderately active disease. The lack of correlation between serum copper concentrations and the AI, number of tender joints and pain may reflect the fact that most of our patients were receiving treatment with non-steroidal anti-inflammatory drugs. These drugs improved joint tenderness and pain, but are thought to have little, if any, effect on the acute phase proteins.<sup>25</sup> Our results, therefore, suggest that serum copper might provide an additional and useful laboratory marker for the assessment of disease activity in RA.

We have shown that IgMRF was significantly correlated with serum copper and patients with IgMRF+ve exhibited significantly higher serum copper concentrations than those with IgMRF-ve. Moreover, patients with extra-articular manifestations showed slightly but not significantly higher median serum copper concentration (29.0  $\mu\text{mol/l}$ ) than those without (24.4  $\mu\text{mol/l}$ ). The presence of both IgMRF and extra-articular features in RA are known to be associated with severe disease.<sup>26</sup> Our finding of highly significant inverse correlation between serum copper concentrations and GS might also be considered as further evidence for the association between raised serum copper and severe disease. Weak grip is mainly the result of either highly active disease or a severely deformed hand and both are manifestations of severe RA.

Oxygen free radicals generated during phagocytosis have been suggested to be responsible for the development of many pathological lesions in RA.<sup>27-30</sup> Furthermore, synovial fluid (SF) copper has been recently shown to correlate significantly with SF free radical oxidation products in patients with RA.<sup>31</sup> Since copper has been shown in vitro to be a good catalyst for free radical reactions,<sup>32</sup> the relevance of raised serum copper to the development of such lesions is of interest.

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Requests for reprints to: Professor DN Baron, Department of Chemical Pathology, Royal Free Hospital, Pond Street, London, NW3 2QG, England.