

# Anaemia in rheumatoid arthritis: the role of iron, vitamin B<sub>12</sub>, and folic acid deficiency, and erythropoietin responsiveness

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## Abstract

Thirty six patients with rheumatoid arthritis (RA) (25 with anaemia) were studied to establish the role of iron, vitamin B<sub>12</sub>, and folic acid deficiency, erythropoietin responsiveness, and iron absorption in the diagnosis and pathogenesis of anaemia in RA. Iron deficiency, assessed by stainable bone marrow iron content, occurred in 13/25 (52%), vitamin B<sub>12</sub> deficiency in 7/24 (29%), and folic acid deficiency in 5/24 (21%) of the anaemic patients. Only 8/25 (32%) had just one type of anaemia. The iron deficiency of anaemia of chronic disease (ACD) was distinguished by ferritin concentration, which was higher in that group. Mean cell volume (MCV) and mean cell haemoglobin (MCH) were lower in both anaemic groups, but most pronounced in iron deficient patients. Folic acid, and especially vitamin B<sub>12</sub> deficiency, masked iron deficiency by increasing the MCV and MCH. Iron absorption tended to be highest in iron deficiency and lowest in ACD, suggesting that decreased iron absorption is not a cause of ACD in RA. No specific causes were found for vitamin B<sub>12</sub> or folic acid deficiency. Haemoglobin concentration was negatively correlated with erythrocyte sedimentation rate in the group with ACD. Erythropoietin response was lower in ACD than in iron deficient patients. It was concluded that generally more than one type of anaemia is present simultaneously in anaemic patients with RA. The diagnosis of each type may be masked by another. Studies on pathogenesis of the anaemia are difficult as deficiencies generally coexist with ACD. Disease activity and, possibly, erythropoietin responsiveness are major factors in ACD pathogenesis.

In patients with active rheumatoid arthritis (RA) anaemia is often present.<sup>1 2</sup> Many types of anaemia are associated with active RA. Vitamin B<sub>12</sub> and folic acid deficiency are reported to be more prevalent among patients with RA than controls.<sup>3 4</sup> The prevalence of iron deficiency is up to 50-70% in RA.<sup>5 6</sup> Many patients with active RA also have the anaemia of chronic disease (ACD) originally described by Cartwright and Lee.<sup>7</sup> Controversial theories exist about the pathogenesis of ACD, including decreased iron absorption,<sup>8 9</sup> iron release by the mononuclear phagocyte system,<sup>10 11</sup> and erythropoietin deficiency.<sup>12 13</sup>

In patients with active RA several causes of anaemia, associated with RA, may be present simultaneously, making studies on diagnosis

and pathogenesis difficult to interpret. We therefore performed a study to assess the prevalence of iron, vitamin B<sub>12</sub>, and folic acid deficiency among patients with RA with and without ACD to determine whether such deficiencies are commonly masked and to establish the role of iron absorption and erythropoietin response in ACD with or without these deficiencies.

## Patients and methods

### PATIENTS

Thirty six patients (five male) with definite or classical rheumatoid arthritis were studied. Patients who had recently been treated with iron, folic acid, or vitamin B<sub>12</sub> were excluded. Patients with a present or past history of ulcers, hypermenorrhoea, haematuria, positive stools for occult blood, haemolysis, or decreased creatinine clearance were also excluded.

Overall disease duration was seven years and 22 (61%) of the patients used longacting anti-rheumatic drugs while 30 (83%) were treated with non-steroidal anti-inflammatory drugs.

Patients with RA were divided into three groups: group I (n=11) consisted of patients without anaemia; group II (n=13) contained anaemic and iron deficient patients; and group III (n=12) consisted of anaemic patients without iron deficiency; they were considered to have ACD (see 'Laboratory procedures'). The mean ages in the groups were group I 54 years, II 62 years, and III 65 years. The difference in age between groups I and III was not significant (p<0.20). No differences in disease duration and antirheumatic drugs used were found in the three groups.

### LABORATORY PROCEDURES

Haemoglobin, mean cell volume (MCV), mean cell haemoglobin (MCH), and reticulocytes were assessed by routine laboratory methods.

Serum iron was measured spectrophotometrically at 520 nm (Instruchemie, Hilversum, The Netherlands), transferrin nephelometrically (Ablon Medical Systems, Leusden, The Netherlands), and ferritin by solid phase enzyme immunoassay (Ferrizyme, Abott Labs, Chicago, USA).

Vitamin B<sub>12</sub> and folic acid were measured by a radioassay technique (Dualcount, Diagnostic Products Corp, Los Angeles, USA).<sup>14 15</sup>

Serum erythropoietin was assessed in 18 serum samples only because of a limited availability of the test by a sandwich radioimmunoassay with monoclonal antibodies. In 30 healthy

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donors mean erythropoietin was 14.5 (SD 4) U/l, which was considered normal.<sup>16</sup>

A Schilling test and assessment of gastric and intrinsic factor antibodies by standard laboratory procedures was performed in patients with low serum vitamin B<sub>12</sub>.

Erythrocyte sedimentation rate (ESR) was measured by the Westergren method and C reactive (CRP) by immunodiffusion (Behring Werke, Marburg, West Germany). A Clq binding assay was carried out by a method originally described by Zubler and Lambert.<sup>17</sup> The Waaler-Rose test was assessed using sensitised sheep erythrocytes<sup>18</sup>; a titre more than 1/32 was considered positive.

The iron absorption test described by Verloop *et al* was used.<sup>19</sup> Serum iron was measured after an overnight fast. A tablet of ferrous sulphate containing 105 mg elementary iron was then ingested, and after two hours serum iron was measured again. The increase in serum iron expressed as a ratio of the final to the initial concentration was taken as an indicator of iron absorption.<sup>19</sup>

Bone marrow was aspirated after sternal puncture in the anaemic patients. Iron content was measured by Perls' Prussian blue. A stainable iron content of 0-1 on a semiquantitative scale was considered as iron deficiency.<sup>20</sup>

#### STATISTICS

Normally distributed data were analysed by Student's *t* test and non-parametric data by the Mann-Whitney U test. Data were correlated using Spearman's coefficient of correlation.

This protocol was accepted by the medical ethical committee of the department of rheumatology of the Dr Daniel den Hoed Clinic.

#### Results

CELLULAR INDICES, MARROW IRON, SERUM VITAMIN B<sub>12</sub>, AND SERUM FOLIC ACID  
Iron deficiency occurred in 13/25 (52%) of anaemic patients (table 1).

Mean cell volume was higher in group I than groups II ( $p < 0.002$ ) and III ( $p < 0.02$ ), while in III it was higher than in II ( $p < 0.05$ ).

Mean cell haemoglobin was higher in I than II ( $p < 0.001$ ) and III ( $p < 0.001$ ). It tended to be higher in III than II ( $p < 0.20$ ).

Reticulocytes were lower in I than II ( $p < 0.05$ ) and III ( $p < 0.02$ ). The difference between II and III was not significant.

Serum vitamin B<sub>12</sub> tended to be lower in group III than in groups I and II, but this was not significant (table 1). Table 2 shows that vitamin B<sub>12</sub> deficiency occurred most commonly in group III, but differences were not significant.

Vitamin B<sub>12</sub> deficiency was present in 10 patients, eight of them (80%) had a normal Schilling test, one (10%) had gastric antibodies, and one (10%) had a gastrectomy.

Haemoglobin concentration was slightly lower in patients without vitamin B<sub>12</sub> deficiency in group II ( $p < 0.20$ ). Mean cell volume was higher in patients with vitamin B<sub>12</sub> deficiency than in those without this deficiency: group I (NS), II ( $p < 0.02$ ), and III ( $p < 0.10$ ). Mean cell haemoglobin was higher in vitamin B<sub>12</sub> deficient patients: group I ( $p < 0.20$ ), II ( $p < 0.02$ ), and III ( $p < 0.05$ ). No correlation existed between serum vitamin B<sub>12</sub> concentration and MCV and MCH except for vitamin B<sub>12</sub> and MCH in the anaemic patients ( $r = -0.36$ ,  $p < 0.05$ ).

Megaloblastic changes in bone marrow were present in four vitamin B<sub>12</sub> deficient patients (40%).

Folic acid deficiency did not occur in any of the non-anaemic patients (group I). In group II it was found in 4/13 (31%) and in group III in 1/11 (9%) patients (table 2). No specific causes of folic acid deficiency were found. Table 1 shows that serum folic acid was lower in groups II and III than in group I ( $p < 0.02$  for both). Mean cell volume and MCH were both higher in patients with folic acid deficiency in groups II and III, but this was not significant. Megaloblastic changes in bone marrow were found in one folic acid deficient patient (20%). In patients with normal vitamin B<sub>12</sub> a low folic acid

Table 1: Erythrocyte variables, iron status, serum vitamin B<sub>12</sub>, serum folic acid, and indices of disease activity in groups I (non-anaemic patients), II (iron deficient patients), and III (patients with the anaemia of chronic disease). Data are expressed as median (range)

	I (n=11)	II (n=13)	III (n=12)
<i>Erythrocyte variables</i>			
Haemoglobin (g/l)	130 (122-135)	97 (68-108)	101 (87-114)
MCV* (fl)	91 (80-97)	78 (71-100)	85 (80-98)
MCH* (pg)	30.4 (26.2-33.3)	25.0 (21.1-32.2)	26.5 (24.5-31.4)
Reticulocytes ( $\times 10^9/l$ )	10 (1-23)	23 (8-44)	32 (13-69)
<i>Iron status</i>			
Serum iron ( $\mu\text{mol/l}$ )	9 (6-12)	4 (1-8)	5 (1-10)
Iron absorption†	1.9 (1.2-5.3)	2.6 (0.8-11.0)	1.7 (0.9-3.5)
Transferrin (g/l)	4.8 (3.8-5.8)	4.4 (2.9-6.0)	3.8 (2.9-4.8)
Ferritin ( $\mu\text{g/l}$ )	31 (10-190)	26 (10-98)	90 (45-221)
Vitamin B <sub>12</sub> (pmol/l)	213 (117-557)	207 (106-415)	165 (38-319)
Folic acid (nmol/l)	13.9 (7.6-26.8)	9.3 (2.9-15.2)	9.0 (5.0-13.9)
<i>Disease activity</i>			
ESR* (mm/h)	36 (14-60)	64 (17-98)	90 (49-128)
CRP* (mg/l)	8 (2-38)	26 (2-117)	36 (6-122)
Clq BA* (%)	5 (3-39)	13 (4-69)	29 (7-100)
Waaler-Rose titre‡	96 (32-512)	256 (64-512)	96 (32-1024)
Number (%) positive	6/11 (54)	8/13 (62)	10/12 (83)

\*MCV=mean cell volume; MCH=mean cell haemoglobin; ESR=erythrocyte sedimentation rate; CRP=C reactive protein; BA=binding assay.

†The rise in serum iron was expressed as a ratio of the final to the initial concentration.

‡Reciprocal titre of Waaler-Rose test. Patients with titres  $> 1/32$  were considered positive.

Table 2: Effect of low serum vitamin B<sub>12</sub> (<150 pmol/l) and low serum folic acid (<7 nmol/l) on cellular indices in non-anaemic patients (I), iron deficient patients (II), and patients with the anaemia of chronic disease (III). Data are expressed as median (range)

	Serum vitamin B <sub>12</sub>		Serum folic acid	
	Low (<150 pmol/l)	Normal (≥150 pmol/l)	Low (<7 nmol/l)	Normal (≥7 nmol/l)
<b>Group I</b>				
Number (%)	3 (30)	7	0	11
Haemoglobin (g/l)	130 (124–135)	127 (122–134)		130 (122–135)
MCV* (fl)	93 (90–97)	90 (80–97)		91 (80–97)
MCH‡ (pg)	31.8 (29.9–34.6)	29.6 (26.2–32.4)		30.4 (26.2–33.3)
<b>Group II</b>				
Number (%)	3 (23)	10	4 (31)	9
Haemoglobin (g/l)	105 (103–106)	95 (68–108)	103 (93–108)	95 (68–108)
MCV (fl)	88 (82–100)	75*** (60–87)	81 (78–87)	77 (60–100)
MCH (pg)	28.1 (25.6–32.2)	24.0*** (21.1–27.9)	25.1 (24.0–26.4)	24.9 (21.1–32.2)
<b>Group III</b>				
Number (%)	4 (36)	7	1 (9)	10
Haemoglobin (g/l)	105 (92–114)	101 (95–113)	106	103 (87–108)
MCV (fl)	90 (80–98)	83* (80–90)	83	85 (80–98)
MCH (pg)	28.3 (25.1–31.4)	25.7** (24.3–28.7)	25.6	26.6 (24.3–31.4)

\*p<0.01; \*\*p<0.05; \*\*\*p<0.02.

†In some patients serum vitamin B<sub>12</sub> or folic acid was not assessed.

‡MCV=mean cell volume; MCH=mean cell haemoglobin.

concentration occurred twice, whereas in patients with normal folic acid concentration low vitamin B<sub>12</sub> was found in three patients.

One patient had a combination of iron, vitamin B<sub>12</sub>, and folic acid deficiency (haemoglobin 106 g/l, MCV 83 fl, MCH 25.6 pg), and another had ACD with folic acid and vitamin B<sub>12</sub> deficiency (haemoglobin 92 g/l, MCV 87 fl, MCH 27.9 pg). After allowance for these findings the differences in cellular indices were slightly larger, but the significance level did not change.

#### MARROW IRON CONTENT AND INDICES OF IRON STATUS

Serum iron concentration was below normal (14 µmol/l) in all patients with RA. It was lower in groups II and III than in group I (p<0.002 for both). The difference between groups II and III was not significant.

Iron absorption was higher in iron deficient patients than in both other groups, but this was not significant. A negative correlation was found between iron absorption and ESR, but this was only significant in group I (r=-0.58, p<0.05). No correlation was found between iron absorption and CRP, C1q binding assay, transferrin, or ferritin. Transferrin was the same in groups I and II, but was lower in III than in I (p<0.01) and II (p<0.05).

Ferritin tended to be lower in group II than in group I (NS). It was higher in group III than in I (p<0.02) and II (p<0.002). A correlation was found between ferritin and ESR when the whole group was considered (r=0.49, p<0.05) as well as CRP (r=0.82, p<0.005 in group II

and r=0.54, p<0.05 in III, while in group I no correlation existed between ferritin and CRP).

#### INDICES OF DISEASE ACTIVITY

Erythrocyte sedimentation rate, CRP, and C1q binding assays were lower in group I than in II and III (p<0.05 at least). They tended to be higher in III than II, but this was in no case significant.

The Waaler-Rose titre tended to be higher in II than I (p<0.10).

Erythrocyte sedimentation rate correlated with haemoglobin only in the group with ACD (r=-0.52, p<0.05). C reactive protein and C1q binding assay did not correlate with haemoglobin. Erythrocyte sedimentation rate correlated negatively with serum folic acid (r=-0.49, p<0.005) as well as CRP (r=-0.43, p<0.01).

#### ERYTHROPOIETIN ASSAYS

Serum erythropoietin was assessed in 18 anaemic patients (12 of them were iron deficient and six had ACD). Table 3 shows that the erythropoietin concentration was higher in iron deficient patients than in patients with ACD (p<0.02). Of the iron deficient patients, one patient (8%) had a serum erythropoietin below normal. One patient in the group with ACD (17%) had a serum erythropoietin below normal. No difference was found between erythropoietin concentrations in patients deficient and non-deficient for vitamin B<sub>12</sub>. Erythropoietin concentration was lower in patients deficient for folic acid (NS).

The erythropoietin concentration correlated

Table 3: Relation between type of anaemia, haemoglobin, and serum erythropoietin. Data are expressed as median (range)

	Iron deficiency*	ACD*†	Low vitamin B <sub>12</sub> ‡	Low folic acid§
Number	9	5	2	2
Haemoglobin (g/l)	95 (68–114)	101 (87–114)	106 (105–106)	103 (93–113)
Erythropoietin (U/l)	60 (10–420)	15 (10–28)	25 (20–30)	20 (10–80)

\*Patients with low vitamin B<sub>12</sub> or folic acid were excluded.

†ACD=anaemia of chronic disease.

‡One patient with iron deficiency, one with anaemia of chronic disease.

§Both patients with iron deficiency.

negatively with haemoglobin concentration ( $r = -0.6$ ,  $p < 0.005$ ) but did not correlate with ESR, CRP, or C1q binding assay. It was found to correlate negatively with ferritin for the whole group ( $r = -0.45$ ,  $p < 0.05$ ) but not in the iron deficient patients ( $r = -0.43$ , NS).

### Discussion

In this study we evaluated the importance of deficiencies of iron, vitamin B<sub>12</sub>, folic acid, and erythropoietin responsiveness and iron absorption in the diagnosis and pathogenesis of anaemia in RA. It was shown that iron deficiency occurred in 13/25 (52%) of anaemic patients based on stainable iron content in bone marrow.<sup>16</sup> In the non-anaemic patients iron deficiency was not ruled out as no marrow aspiration was performed in this group. Anaemia of chronic disease (normal to increased stainable bone marrow iron) occurred in 12/25 (48%).

Vitamin B<sub>12</sub> deficiency occurred in about 30% of patients in all three groups (serum vitamin B<sub>12</sub> lower than 150 pmol/l) and folic acid deficiency (serum folic acid < 7 nmol/l) was present in 24% of anaemic patients (31% in group II and 9% in group III).

### DIAGNOSTIC ASPECTS

Mean cell volume and MCH were found to be lower in the iron deficient patients than in the non-anaemic patients. A hypochromic microcytic anaemia is often found in iron deficient patients without chronic disease.<sup>21</sup> In our study MCV and MCH were also lower in anaemic patients with RA without iron deficiency (considered as patients with ACD), though they were higher than in iron deficient patients. Other studies also reported a lower MCV and MCH<sup>2 7 22</sup> in patients with ACD.

Anaemia caused by iron deficiency is not easily distinguished from ACD in patients with active RA by means of MCV and MCH. Specificity of the MCV in RA is reported to be high but the sensitivity low.<sup>5 18</sup>

Reticulocytes were found to be higher in both anaemic groups, suggesting some response to the anaemia. They tended to be higher in the group with ACD, but this was not significant. No difference in reticulocyte count was found between those with vitamin B<sub>12</sub> and folic acid deficiency and those with other types of anaemia.

When the differences in cellular indices in iron deficiency and ACD are evaluated it should be remembered that these differences may be slightly masked because of the coexistent presence of vitamin B<sub>12</sub> or folic acid deficiency. Vitamin B<sub>12</sub> deficiency was the same in all three groups, whereas folic acid deficiency was found more often in iron deficient patients.

Vitamin B<sub>12</sub> deficiency has an important influence on cellular indices, which is most pronounced in iron deficient patients. Patients with a combination of iron and vitamin B<sub>12</sub> deficiency had a normochromic normocytic anaemia. Thus reliance on cellular indices would have led to many patients being classified as having ACD, in which normal cellular

indices are commonly found.<sup>6 7 19</sup> Vitamin B<sub>12</sub> deficiency is usually associated with a hyperchromic macrocytic anaemia.<sup>23</sup> Iron deficiency is associated with a hypochromic microcytic anaemia<sup>17</sup> so the combination results in normal indices. Folic acid deficiency exerted the same effects on cellular indices, but these were not significant.

Serum iron concentration was below normal in all patients, including the non-anaemic patients, though it was lower in both anaemic groups. It is known that serum iron does not distinguish between iron deficiency and ACD,<sup>7 21</sup> which implies that it does not correlate with body iron stores. The low serum iron in non-anaemic patients might be explained by the fact that in chronic disease, to some extent also present in this group, serum iron is low, possibly through trapping by ferritin<sup>21</sup> or the mononuclear phagocyte system.<sup>10 11</sup>

It was shown that in most patients with RA several causes of anaemia were present simultaneously. We assume that iron deficient patients also have features of ACD as a negative correlation was found between haemoglobin concentration and disease activity (ESR) in the group with ACD, and indices of disease activity were higher in iron deficient patients than in non-anaemic patients. This assumption was confirmed by the lower transferrin concentration in iron deficient patients than in non-anaemic patients, though ferritin concentration was not low in either non-anaemic or iron deficient patients, but transferrin concentration was lowest and ferritin concentration highest in patients with ACD. Ferritin behaves like an acute phase reactant<sup>24 25</sup> as it correlates with ESR and CRP. Among patients with ACD iron deficiency can be detected most easily by means of the lower ferritin concentration, though it is not subnormal. Other studies have suggested raising the cut off point to 60 µg/l<sup>5</sup> to detect iron deficiency.

The dichotomy ACD versus iron deficiency, therefore, is artificial because in fact it is a combination. Thus we found that only 8/25 (32%) anaemic patients with RA had just one cause of anaemia (ACD).

All other patients had combinations of iron, vitamin B<sub>12</sub>, or folic acid deficiency, with or without ACD. In two patients (8%) both folic acid and vitamin B<sub>12</sub> deficiency occurred. This implies not only that accurate diagnostic attention should be paid to the approach of anaemic patients with RA but also that several therapeutic possibilities in the treatment of anaemia in RA are present apart from treating the RA.

### PATHOGENETIC ASPECTS

#### *Iron deficiency*

We found iron deficiency in 13/25 (52%) of anaemic patients with RA, which is in agreement with findings of other authors, who found a prevalence of 50–70%.<sup>5 6</sup> Although in our study patients with gastrointestinal disease and positive stools for occult blood were excluded, gastrointestinal lesions and blood loss can occur subclinically and intermittently.<sup>26 27</sup> It is important to rule out gastrointestinal blood loss

in patients with RA. It was recently found that cellular indices and ferritin did not differ in anaemic patients with RA with or without gastrointestinal lesions.<sup>28</sup> Thus selection of anaemic patients with RA for gastrointestinal tract investigation remains difficult.

In our study iron absorption measured by a simple, non-invasive test tended to be higher in iron deficient patients with RA than in non-anaemic patients and patients with ACD. It correlated negatively with indices of disease activity, particularly in the non-anaemic group. Other authors, using radiolabelled <sup>59</sup>Fe absorption studies, showed that iron absorption is lower in active RA. Iron absorption in iron deficient patients with active RA is higher than in those with ACD but lower than in iron deficient patients without chronic disease.<sup>9 29</sup> It may be concluded that decreased iron absorption is the result of active RA and not a cause of ACD or iron deficiency in RA.

#### *Vitamin B<sub>12</sub> deficiency*

We found vitamin B<sub>12</sub> deficiency in 7/24 (29%) of the patients with RA. As the prevalence was the same in non-anaemic patients its role as a cause of anaemia in RA becomes less clear. It was recently shown, however, that vitamin B<sub>12</sub> deficiency can be present without anaemia or the typical change in indices,<sup>30</sup> but this was found in a minority of vitamin B<sub>12</sub> deficient patients. Overall serum vitamin B<sub>12</sub> tended to be lower in the anaemic patients, particularly in the patients with ACD, median vitamin B<sub>12</sub> concentration being just above the lower limit (150 pmol/l). Schilling tests were normal in most patients, which was also found by Couchman *et al.*<sup>3</sup> We found a normal prevalence of gastric antibodies and pernicious anaemia in our patients, in contrast with others,<sup>3 31</sup> who found an increased prevalence. This difference is probably due to the small number of patients studied here. Thus vitamin B<sub>12</sub> deficiency is high among patients with RA, but its cause remains to be established.

#### *Folic acid deficiency*

Folic acid deficiency was seen most often in iron deficient patients. Serum folic acid concentration was significantly lower in the anaemic than in the non-anaemic patients. Cough *et al* found a low serum folic acid concentration in 65% of the patients with RA (cut off point 6 nmol/l).<sup>32</sup> In that study no specific causes were found either. The coexistence with iron deficiency suggests either malabsorption, which was not found, or dietary causes. We found a negative correlation between serum folic acid, ESR and CRP, suggesting lower concentrations in active RA. It is not entirely clear whether low serum folic acid has a causative role in ACD or whether active RA, associated with ACD, produces a secondary low serum folic acid concentration as folic acid concentration was normal in non-anaemic patients. Anorexia due to active RA or increased use by proliferating synovial cells<sup>32 33</sup> may be a possible explanation.

#### *Disease activity*

Erythrocyte sedimentation rate, CRP, and C1q

binding assay were lowest in non-anaemic patients. In iron deficient patients disease activity was intermediate and it was highest in ACD. We found that ESR correlated negatively with haemoglobin only in the group with ACD. From these findings it may follow that in ACD, particularly, the haemoglobin concentration is dictated by RA activity and that anaemia is mostly seen in active RA. Birgegard *et al* also found a correlation between ESR and haemoglobin.<sup>12</sup> The Waaler-Rose titre did not correlate with the other indices of disease activity.

Rheumatoid arthritis in iron deficient patients was less active than in ACD. It could be speculated that this is caused by the iron deficiency itself as it is known that iron deficiency may be beneficial in RA synovitis,<sup>34</sup> and disease activity might increase after iron supplementation.<sup>35</sup> It could also be argued that if these patients had a higher level of disease activity iron trapping by the mononuclear phagocyte system<sup>10 11</sup> would have resulted in a decreased iron metabolism and hence disappearance of iron deficiency.

#### *Erythropoietin deficiency*

The erythropoietin concentration was significantly lower in patients with ACD than in iron deficient patients, whereas haemoglobin did not differ significantly between these groups. As stated before these two causes of anaemia cannot be fully separated in patients with RA, and thus the difference of erythropoietin concentrations in these groups might have been less. Patients with low vitamin B<sub>12</sub> or folic acid had lower erythropoietin concentrations but they were equally distributed between the iron deficient group and the group with ACD, which, together with the small number of patients involved, makes interpretation of this finding difficult. A negative correlation was found between haemoglobin and erythropoietin, most pronounced in iron deficient patients. In both groups only one patient had a serum erythropoietin below normal (1/12 (8%) among iron deficient patients and 1/16 (17%) in those with ACD). Erythropoietin correlated negatively with ferritin. These findings suggest that the erythropoietin response to anaemia caused by iron deficiency in RA is higher than to ACD. Baer *et al* also found lower erythropoietin concentrations in ACD in RA.<sup>36</sup> Birgegard *et al* and Erslev *et al*, however, found higher erythropoietin concentrations in anaemic patients with RA than in non-anaemic controls irrespective of the cause of anaemia.<sup>12 13</sup> Patients with ACD showed lowest erythropoietin and highest ESR, CRP, and C1q binding assay, though no correlation was found between ESR and erythropoietin in contrast with the results of Birgegard *et al.*<sup>12</sup> Possibly, therefore, a factor that correlated with disease activity might have had a restrictive effect upon erythropoietin production. The definite role of decreased erythropoietin responsiveness in the pathogenesis of ACD could be established by treating anaemic RA patients with exogenous recombinant DNA erythropoietin, after which the response should be evaluated for the various causes of anaemia in RA.

It is concluded that as more than one cause of anaemia was usually found in patients with RA in this study, findings from studies on the pathogenesis of anaemia in RA should be interpreted with caution. The various dichotomies used here are artificial to some extent. Thus iron deficient patients also have features of ACD because they usually have active RA. Disease activity and, possibly, decreased erythropoietin responsiveness are major factors in the pathogenesis of ACD.

In summary, we investigated the role of iron, vitamin B<sub>12</sub>, and folic acid deficiency as well as iron absorption and erythropoietin responsiveness in 36 patients with RA in order to establish their role in anaemia. All deficiencies were found to be highly prevalent in anaemic patients with RA. Cellular indices were unreliable in the differential diagnosis as iron deficiency was marked by vitamin B<sub>12</sub>, and to a lesser extent, by folic acid deficiency, whereas MCV and MCH were lower in both iron deficiency and ACD. Ferritin is useful in detecting iron deficiency among patients with ACD. Decreased iron absorption does not have a role in the pathogenesis of iron deficiency or ACD. In ACD disease activity and possibly decreased erythropoietin responsiveness are factors involved, but one should be cautious about the interpretation because patients with RA were usually found to have more than one type of anaemia simultaneously; only 8/25 (32%) of patients had just one type of anaemia (ACD).

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