Rheumatol Int (2004) 24: 14–19
DOI 10.1007/s00296-003-0323-2

ORIGINAL ARTICLE

**Refael Segal · Yehuda Baumoehl · Ori Elkayam
David Levartovsky · Irena Litinsky
Daphna Paran · Irena Wigler · Beni Habot
Arthur Leibovitz · Ben Ami Sela · Dan Caspi**

Anemia, serum vitamin B12, and folic acid in patients with rheumatoid arthritis, psoriatic arthritis, and systemic lupus erythematosus

Received: 1 October 2002 / Accepted: 25 February 2003 / Published online: 29 April 2003

Abstract *Objective*: Although anemia is frequent in inflammatory rheumatic diseases, data regarding vitamin B12 status is scarce. The purpose of this study was to analyze the incidence and nature of B12 and folic acid (FA) deficiencies in a cohort of rheumatic patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), and systemic lupus erythematosus (SLE). *Methods*: Levels of B12, FA, and parameters of anemia were recovered or examined in 276 outpatients. In those with recent findings of low serum B12 levels, further studies of serum homocysteine (Hcy) and urine methylmalonic acid (MMA) levels were performed. *Results*: The incidence of anemia was high: 49%, 46%, and 35%, in RA, SLE, and PsA, respectively. Low levels of serum B12 were also frequent (24%), with almost similar occurrence in the three disease groups. Deficiency in FA was rare ($< 5\%$). Mean levels of both vitamins did not differ significantly among the three groups. No correlation between serum B12 levels and anemia was found. In the 15 patients with recently detected low B12 levels, Hcy and MMA were evaluated before and following B12 therapy. In ten of them, baseline Hcy levels were high, while MMA was increased in one patient only. Response to B12 administration, i.e., a decrease in Hcy and/or MMA levels, was noticed in four patients only, suggesting that only 26% of the low-serum-B12 patients had true B12 deficiency. *Conclusions*: The incidences of anemia and decreased serum B12 levels were high in these three groups of rheumatic patients. However, true tissue deficiency seems to be much rarer.

Keywords Folic acid · Psoriatic arthritis · Rheumatoid arthritis · Systemic lupus erythematosus · Vitamin B12

R. Segal (✉) · Y. Baumoehl · B. Habot · A. Leibovitz
Department of Geriatrics, Shmuel-Harofeh Geriatric Medical Center, Baer Yaakov, Israel
E-mail: shmuelh@netvision.net.il
Fax: +972-8-9237156

O. Elkayam · D. Levartovsky · I. Litinsky · D. Paran
I. Wigler · D. Caspi
Rheumatology Department, Sourasky Medical Center, Tel Aviv, Israel

B. A. Sela
Department of Chemistry, Sheba Medical Center, Tel Hashomer, Israel

R. Segal · Y. Baumoehl · O. Elkayam · D. Levartovsky
I. Litinsky · D. Paran · I. Wigler · B. Habot · A. Leibovitz
B. A. Sela · D. Caspi
Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction

A tendency to develop deficiencies in vitamin B12, folic acid (FA), vitamin B6, and iron has been suggested in patients with chronic rheumatic diseases [1, 2, 3, 4, 5, 6]. Deficient nutrition and eventual malabsorption secondary to autoimmune mechanisms are the possible culprits [5, 7, 8]. Moreover, an inverse correlation between B12 levels and duration and activity of the rheumatic disease has been reported [2, 3]. In recent years, increased cardiovascular morbidity among rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients has been highlighted and related to mechanisms of ongoing inflammatory and autoimmune processes [9, 10] as well as to a tendency of severe RA and SLE patients to develop hyperhomocysteinemia [3, 11, 12], an additional and independent risk factor for cardiovascular disease [13, 14, 15].

Since hyperhomocysteinemia may result from B12 and FA deficiency [14, 16, 17], we initiated this study with the aim of assessing the incidence of these deficiencies in rheumatic patients with the chronic inflammatory disorders RA, SLE, and psoriatic arthritis (PsA). The literature covering this subject is limited and mainly concerns serum levels of the vitamins [1, 2, 6, 12, 18, 19]. These levels, however, do not reliably reflect vitamin B12 status. Thus, in order to detect true tissue B12 deficiency,

we aimed to evaluate homocysteine (Hcy) and methylmalonic acid (MMA) levels due to the well known inverse correlation between these and tissue B12 [16]. To the best of our knowledge, only a single prior work by Pettersson et al. [3] attempted to assess B12 deficiency in RA patients through Hcy and MMA evaluation, while no such studies were published in patients with SLE and PsA. Since folate deficiency is not infrequent among rheumatic patients [2] and constitutes an important cause of hyperhomocysteinemia [14, 16], folate status was also evaluated.

Finally, given the connection between B12, folate deficiency, rheumatic diseases and anemia [1, 2, 6], we used the data to evaluate the incidence and characteristics of anemia in these patients.

Materials and methods

Patients

Two hundred one patients with RA, 35 with SLE, and 40 with PsA according to the currently accepted American College of Rheumatology criteria [20], participated consecutively in this study. It consisted of a retrospective search of file data (156 patients), prospective interviews and examinations, followed by assessment of current vitamin B12 and folate serum levels (120 patients). Complete blood count, red blood cell parameters, vitamin B12, and folic acid were available in all 276 patients. Iron and ferritin levels were measured only in the prospective cohort of patients (118 out of 120). Of these, 89 had RA, 14 PsA, and 15 SLE. Dietary history was recovered from 193 patients. In those with newly detected low serum vitamin B12, baseline Hcy and MMA levels were measured. This was followed by IM injection of 1,000 μg of vitamin B12 twice weekly for 2 consecutive weeks. One month later, complete blood cell count and B12, Hcy, and MMA levels were reassessed.

Laboratory measurements

Samples of patients' serum and urine were immediately stored at −20°C for short-term storage. Serum B12, folate, and ferritin levels were measured through electrochemiluminescence immunoassay (Eclesys 2010 Analyzer) (Roche Diagnostics, Indianapolis, Ind., USA). Normal values are 220–1,300 ng/L for B12, 3–17 mg/ml for folate and 14–150 ng/ml for ferritin. Iron was measured by means of the photometric color test, TPTZ method, Olympus system reagent (ALL-200 analyzer) (Olympus Diagnostica, Hamburg, Germany). Normal values are 35–150 μmol/L. Levels of Hcy were measured by fluorescence detection as described by Jacobsen et al. [17]. This method measures the concentrations of free, protein-bound, and mixed disulfide Hcy. Normal values range between 5.0 μmol/L and 15.0 μmol/L. Methylmalonic acid in urine was measured by capillary gas chromatography and mass spectrometry according to Stabler [21]. Values of < 3.0 mg/g creatinine are considered normal.

Statistical methods

Statistical analyses were performed using statistical software (SPSS, Chicago, Ill., USA) using Student's *t*-test, analysis of variance, Pearson's correlation coefficients, and the chi-squared test.

Results

Demographic data and clinical characteristics of the patients are reported in Table 1. Their various antirheumatic treatment regimens are summarized in Table 2. Data on other factors that may influence B12 and FA levels, i.e., diet, supplements, and drugs, were available in 193 patients. Of them, 5.2% were strict vegetarians, all in the RA group. H_2 blockers or proton pump inhibitors were used by 11% and 3% of the patients, respectively, and 9.4% of the patients consumed multivitamins. Folate supplementation was used by 12.3% of patients (Table 3).

Serum B12 levels were normal and very similar in the three disease groups (mean 358 ± 212 ng/L). The total incidence of low serum B12 was 23.6% (65 patients), without significant differences between the three disease groups (Table 4). The mean level in this group was 157 ± 34 ng/L vs 419 ± 206 ng/L in the remaining 211 subjects. While cobalaminopenic SLE patients had but very mild reductions in serum B12 levels (180–190 ng/L), the reductions were much more significant in half of the RA and PsA patients (< 160 ng/L).

In the attempt to characterize low B12 patients, several demographic, clinical, and laboratory parameters were evaluated (Table 5). Vegetarianism was the only factor found to be consistently associated with low B12, with a 10.5% incidence among low B12 patients vs 2.9% among normocobalaminic subjects ($P = 0.04$, chi-squared test). Sixty percent of the vegetarian patients had low serum B12 vs 28% (51 out of 183) of the non-vegetarians. Additional clinical and laboratory parameters such as age, disease duration, incidence of anemia, hemoglobin level, and mean corpuscular volume (MCV) did not differ significantly between low- and normal-serum-B12 patients. No significant correlation was found between hemoglobin or MCV values and serum B12 levels. Concomitant low FA was present in only four of the 65 patients with low B12.

Fifteen of the 65 patients with recent findings of low serum B12 during the study period were naive to vitamin B12 supplementation. One of them had PsA, while 14 had RA. In this group, baseline Hcy and MMA

Table 1 Demographic data

	RA	PsA	SLE	Total
N (%)	201 (72.8%)	40 (14.5%)	35 (12.5%)	276
Mean age (range)	60.4 ± 15 (22–92)	54 ± 16 (23–87)	37.6 ± 13 (19–59)	56.6 ± 16.6 (19–92)
Mean disease duration in years (range)	10.94 (1–45)	12.60 (2–41)	7.37 (1–22)	10.7 (1–45)
N male	47	20	4	71 (25.7%)
N female	154	20	31	205 (74.3%)

Table 2 Concomitant antirheumatic treatment.*SSZ* sulfasalazine, *HCQ* hydroxychloroquine, *MTX* methotrexate

	RA ($n=201$)	PsA ($n=40$)	SLE ($n=35$)	Total ($n=276$)
Prednisone	77 (38%)	2 (5%)	22 (63%)	101 (37%)
MTX	146 (73%)	28 (70%)	3 (9%)	177 (65%)
SSZ	14 (7%)	6 (15%)	0	20 (7%)
Minocyclin	12 (6%)	1 (2.5%)	0	13 (4%)
Gold	33 (16%)	3 (7.5%)	0	36 (13%)
Azathioprin	6 (3%)	1 (2.5%)	6 (17%)	13 (4%)
HCQ	39 (19%)	0	23 (66%)	62 (23%)

Table 3 Incidence of factors with possible influence on B12 or folate levels. *PPI* proton pump inhibitors

	RA (201)	PsA (40)	SLE (35)	Total (276)
Vegetarian diet[a]	10 /133 (7.5%)	0/25	0/35	10/193 (5.2%)
H2 blockers	25 (12.5%)	5 (12.5%)	2 (5.7%)	32 (11%)
PPI	4 (2%)	2 (5%)	4 (11.4%)	10 (3%)
Multivitamins	24 (12%)	0	2 (6%)	26 (9.4%)
Folic acid	24 (12%)	4 (10%)	6 (17%)	34 (12.3%)

[a]Based on available data of 193 patients

measurements (Table 6) showed high Hcy levels (>15 μmol/L) in ten patients (67%), all having normal serum folate levels, while elevated MMA was present in only a single patient who had normal levels of Hcy. These 15 patients were treated twice weekly with 1,000-μg B12 injections for 2 weeks, and their B12, Hcy, and MMA levels were retested after 4 weeks. This was followed by a two- to fourfold increase in B12 serum levels. The elevated baseline Hcy levels in ten patients, normalized in three, while the high baseline MMA observed in one case also normalized. These results suggest that true vitamin B12 deficiency was present in only 26% (4/15) of the recently detected low serum B12 level patients.

Mean serum FA levels were within the normal range (10.4 ± 8.6 mg/ml) and similar in the various groups (Table 4). The incidence of low serum FA levels in the whole cohort was found to be very low (4.4%). It was present mainly in RA patients (5%) and less in the PsA group (2.5%). None of the SLE patients had low serum

Table 5 Characteristics of B12-deficient rheumatic patients

	B12-deficient (<200 ng/L) ($n=65$)	B12 >200 ng/L ($n=211$)
Mean age (years)	55.6 ± 15.4	56.9 ± 17
Mean B12 level	157.1 ± 33.6	419.5 ± 206
Disease duration (years)	11.8 ± 9.3	$10.4y\pm8.3$
Vegetarian diet[a]	6 (10.5%)*	4 (2.9%)
Hb	12.5 ± 1.3	12.4 ± 1.3
Anemia as Hb <12 g/dL	28 (43%)	100 (47%)
Mean MCV	87 ± 5.9	86 ± 7.3
High MCV level	4 (6.2%)	14 (6.7%)
Low MCV level	6 (9.2%)	32 (15.2%)

[a]Data available in 193 patients
*$P<0.05$ (chi-squared test)

folic acid levels. A similar distribution of low or normal serum folic acid levels was found in patients on methotrexate (MTX) therapy compared to patients on other drugs. None of the low serum folate patients received multivitamins or folic acid supplementation.

Mean Hb levels were 12.5 ± 13 g/dL in the entire cohort and almost identical in the three disease groups (Table 4). However, the incidence of anemia (Hb4).

Mean iron levels (Table 4) were within the normal range and almost identical in the various groups (60.2 ± 29.3 μmol/L). Low serum iron levels were detected in 22% of the patients, without significant intergroup differences. In contrast, ferritin levels did differ among the study groups. The incidence of low serum ferritin was significantly higher in SLE patients than the RA and PsA groups (40% vs 11% and 14%, respectively, $P=0.01$). Similarly, the mean values of ferritin were lower among SLE patients (47 ± 54 ng/ml vs 88 ± 168 ng/ml in RA and 125 ± 182 ng/ml in PsA), although not reaching statistical significance.

Finally, characterization of the anemia based on the available laboratory results of B12, FA, and partial data on iron and ferritin (118 patients of the prospective cohort) shows a similar distribution of the three common types of anemia: iron deficiency in 21% of anemic patients (low ferritin), anemia of chronic disease in 22% (low iron and normal or high ferritin), and B12 deficiency in 22.7% of anemic subjects. Of the remaining anemic patients,

Table 4 Laboratory data

	RA	PsA	SLE	Total
Mean Hb (range)	12.3 ± 1.3 (9–16)	13 ± 1.5 (10–17)	12.4 ± 1.4 (9.5–15)	12.5 ± 1.3 (9–17)
Anemia (Hb <12 g/dL)	98/201 (49%)	14/40 (35%)	16/35 (46%)	128/276 (46.4%)
Mean MCV (range)	86 ± 7.3 (61–111)	85.8 ± 5.5 (76–101)	86.9 ± 6.9 (67–97)	86.1 ± 7.0 (61–111)
Mean serum B12 in ng/L (range)	358 ± 214 (66–1258)	341 ± 185 (89–1054)	372 ± 238 (178–1500)	358 ± 212 (66–1500)
Decreased B12 (<200 ng/L)	50/201 (24.9%)	8/40 (20%)	7/35 (20%)	65/276 (23.6%)
Mean serum folate in mg/ml (range)	10.5 ± 8.9 (2.1–45)	8.8 ± 9.1 (2.8–45)	11.7 ± 5.6 (4.0–22)	10.4 ± 8.6 (2.1–45)
Decreased folate (<3 mg/ml)	11/201 (5%)	1/40 (2.5%)	0	12/276 (4.4%)
Mean iron in μmol/L (range)[a]	60.1 ± 31 (8–153)	55.8 ± 17 (30–81)	63.4 ± 28.5 (7–123)	60.2 ± 29.3 (7–153)
Decreased iron as <35 μmol/L	17/74 (23%)	2/11 (18%)	3/16 (19%)	22/101 (21.8%)
Mean ferritin in ng/ml (range)[a]	88.3 ± 168 (1.7–1476)	125 ± 182 (7.0–714)	47 ± 54 (3.2–183)	87.4 ± 160 (1.7–1476)
Decreased ferritin as <14 ng/ml	10/89 (11%)	2/14 (14%)	6/15 (40%)*	18/118 (15.3%)

[a]Measured in 118 patients
*P <0.01 vs RA and PsA

Table 6 Homocysteine (Hcy) and methylmalonic acid (MMA) levels in 15 rheumatic patients with low serum B12

Level	Mean Hcy in μmol/L	*N* with high[b] Hcy (%)	Mean MMA as mg/g creatinine	*N* with high MMA[c]
Baseline (range)	18.7 ± 7.2 (9–32)	10/15 (67%)	0.9 ± 1.2 (range 0–4)	1/15 (6.6%)
After B12 treatment (range)[a]	15.5 ± 7.8 (5–28)	7/15 (46%)	0.9 ± 6.6 (0.5–2)	0

[a] 4 weeks after B12 therapy (i.m. 1000 μg×4)
[b] > 15 μmol/l)
[c] > 3 mg/g creatinine

about 31% had mixed types of anemia (Table 7). The incidence of low serum ferritin and the combination of low iron with normal or elevated ferritin were found to be significantly higher in patients with anemia (of iron deficiency and of chronic disease, respectively) than in nonanemic subjects ($P < 0.05$). In contrast, B12 and folate serum levels did not correlate with anemia. The incidence of B12 and folate deficiencies was in fact somewhat higher in nonanemic than in anemic patients (26% and 5% vs 23% and 3%, respectively).

The inclusion of two patient cohorts (retrospective and prospective) may be considered a limitation of this study. However, the fact that no differences between the two groups were noted in the distribution of deficiencies of any of the examined data (anemia, B12, FA) lends support to their combined consideration.

Discussion

It is commonly assumed that patients with chronic rheumatic diseases tend to develop multiple vitamin and mineral deficiencies, vitamin B12 and folic acid among them [1, 2, 3, 4, 5]. Seemingly, an inverse correlation exists between B12 levels and duration and activity of the rheumatic disease [2, 3]. These deficiencies may be related to impaired nutrition and, to a certain extent, malabsorption. Drug therapy may take its toll, too. Thus, nonsteroidal anti-inflammatory drugs (NSAIDs) may enhance gastrointestinal blood loss, while H_2 blockers or proton pump inhibitors may contribute to B12 and iron deficiency by interfering with absorption, and MTX and/or sulfasalazine (SSZ) may influence folinic or folate status [22]. Combined deficiencies can contribute to the manifestation of anemia, per se a common accompaniment of inflammatory rheumatic diseases. Anemia is mostly seen in active and protracted disease, reaching an incidence as high as 70% [2]. Anemias of chronic disease and/or iron deficiency are the most common types, while B12 and folate deficiencies are less frequent causes of anemia [1, 2, 6].

Table 7 Characteristics of anemia in rheumatic patients. Iron and ferritin data were available in only 118 patients of the prospective cohort, and hence the statistics concern only this subgroup of patients. *ACD* anemia of chronic disease

	Anemia (Hb < 12)	No anemia (Hb > 12)
Iron deficiency (low ferritin)*	21%	9%
ACD (low iron and normal-to-high ferritin)*	22.5%	13.5%
B12 deficiency	22.7%	25.7%
Folic acid deficiency	3.1%	5.4%
Mixed causes of anemia	30.7%	

*$P < 0.05$ (chi-squared test)

Of the 276 rheumatic patients in our study, the majority had RA, while 40 and 35 had SLE or PsA, respectively. Most had long-standing disease. About 24% of the patients were found to have low serum B12. Significantly low levels were present only in RA and PsA patients, while SLE patients had only very mildly reduced serum levels. The incidence of low serum B12 mentioned in the literature ranges from 4% in Pettersson's large survey of 1,000 RA patients [3] to 30% in Vreugdenhil's study of 36 patients [2]. Strict vegetarianism or use of drugs that impair B12 absorption was relatively rare. The former, however, was found in our study to accompany low B12 levels consistently (deficiency incidence of 60% among vegetarians).

In the subgroup of patients with recent low B12 detection, elevated baseline Hcy was high (66%), while increased MMA was seen in a single patient (< 7%). However, based on the response to B12 administration, true B12 deficiency could be confirmed in only 33% of the patients with increased Hcy and altogether in only 26% of the low-serum B12 patients studied in this regard. A similar percentage was found by Pettersson [3]. The relatively high incidence of elevated Hcy detected in our patients is in accordance with the known tendency of long-standing RA and other chronic inflammatory states to develop hyperhomocysteinemia [3, 11, 12]. In most of these patients, hyperhomocysteinemia was not corrected by the addition of B12, which suggests that low levels of B12 do not play an important role in the pathophysiologic mechanism of hyperhomocysteinemia in this cohort of rheumatic patients. The incidence of anemia was not increased in the subgroup of low-B12 patients, confirming the well-known fact that decreased levels of B12 are often accompanied by normal levels of hemoglobin [23, 24, 26]. Increased MCV values were also a rarity in this group, as previously reported [25, 27]. The frequent concomitant iron deficiency in rheumatic patients could be one possible explanation.

Folic acid deficiency was found to be relatively rare (< 5%), in spite of the high percentage of patients on MTX/SSZ therapy (73%) and the relatively small pro-

portion of FA supplementation. Vreugdenhil's study, with a similar percentage of patients on MTX/SSZ but no FA supplementation, reported a 15% overall incidence of FA deficiency [2].

In our cohort, the incidence of anemia was high, reaching 45%. In Vreugdenhil's study [2] it was even higher (70%). Based on our partial evaluation (without bone marrow aspiration or measurement of transferrin receptor status), we found even distribution of iron, B12 deficiency, and anemia of chronic disease (21–23%).

Attempting to correlate the incidence of anemia with the various hematologic parameters, only low ferritin and the combination of low iron with normal or high ferritin were found to constitute reliable predictors of anemia (of iron deficiency and of chronic disease, respectively). Surprisingly, low B12 and folate levels were in fact somewhat more common in our nonanemic patients.

The present work is the first to compare certain aspects of anemia, its parameters, and B12/folate/ferritin among these three groups of rheumatic patients. Our results show that SLE patients differed in certain aspects from RA/PsA subjects. They were younger, with an obvious female predominance (8:1). When present, B12 deficiency was but borderline, and none of them had folate deficiency. In contrast, mean ferritin tended to be lower in SLE patients, and their incidence of decreased ferritin was the highest ($P = 0.01$). The higher ferritin levels among RA and PsA patients may be, however, related to a more pronounced acute phase reaction in these two chronic inflammatory diseases.

In conclusion, our study evidences a relatively high incidence of decreased serum B12 levels in rheumatic patients. Apparently, however, true B12 deficiency is much less frequent. Thus, the clinical significance of low serum B12 was found to be of limited value except for screening in the ongoing challenge to identify vitamin B12 deficiency at a stage when treatment can still avert irreversible psychoneurologic damage [25, 26, 27].

References

1. Vreugdenhil G, Lindemans J, Eijk HG van, Swaak AJ (1992) Elevated serum transcobalamin levels in anaemia of rheumatoid arthritis: correlation with disease activity but not with serum tumour necrosis factor alpha and interleukin 6. J Intern Med 231:547–550
2. Vreugdenhil G, Wognum AW, Eijk HG van, Swaak AJ (1990) Anaemia in rheumatoid arthritis: the role of iron, vitamin B12, and folic acid deficiency, and erythropoietin responsiveness. Ann Rheum Dis 49:93–98
3. Pettersson T, Friman C, Abrahamsson L, Nilsson B, Norberg B (1998) Serum homocysteine and methylmalonic acid in patients with rheumatoid arthritis and cobalaminopenia. J Rheumatol 25:859–863
4. Grindulis KA, Calverley M, Cox C (1984) Rheumatoid arthritis: is serum vitamin B12 high in active disease? J Rheumatol 11:211–212
5. Roubenoff R, Roubenoff RA, Selhub J, Nadeau MR, Cannon JG, Freeman LM, Dinarello CA, Resenberg IH (1995) Abnormal vitamin B6 status in rheumatoid cachexia. Association with spontaneous tumor necrosis factor alpha production and markers of inflammation. Arthritis Rheum 38:105–109
6. Giordano N, Cecconami L, Marcucci P, Battisti E, Magaro L, Morozzi G, Mariano A, Marcolongo R (1992) The role of iron, vitamin B12, folic acid and erythropoietin in the anemia of rheumatoid arthritis. Clin Exp Rheumatol 10:201–202
7. Dyer NH, Kendall MJ, Hawkins CF (1971) Malabsorption in rheumatoid disease. Ann Rheum Dis 30:626–630
8. Benn HP, Drews J, Randzio G, Jensen JM, Loffler H (1988) Does active rheumatoid arthritis affect intestinal iron absorption? Ann Rheum Dis 47:144–149
9. Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, Rosenberg IH (1996) Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet 348:1120–1124
10. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM, Cathey MA (1994) The mortality of rheumatoid arthritis. Arthritis Rheum 37:481–494
11. Roubenoff R, Dellaripa P, Nadeau MR, Abad LW, Muldoon BA, Selhub J, Rosenberg IH (1997) Abnormal homocysteine metabolism in rheumatoid arthritis. Arthritis Rheum 40:718–722
12. Hernanz A, Plaza A, Martin-Mola, De Miguel E (1999) Increased plasma levels of homocysteine and other thiol compounds in rheumatoid arthritis women. Clin Biochem 32:65–70
13. Graham IM, Daly LE, Refsum HM (1997) Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 277:1775–1781
14. Jacobsen DW (1998) Homocysteine and vitamins in cardiovascular disease. Clin Chem 44:1833–1843
15. Pancharuniti N, Lewis CA, Sauberlich HE, Perkins LL, Go RC, Alvarez JO, Macaluso M, Acton RT, Copeland RB, Cousins AL (1994) Plasma homocysteine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. Am J Clin Nutr 59:940–948
16. Klee GG (2000) Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs. vitamin B12 and folate. Clin Chem 46:1277–1283
17. Jacobsen DW, Gatautis VJ, Green R, (1994) Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 40:873–881
18. Leeb BF, Witzmann G, Ogris E, Studnicka-Benke A, Andel I, Schweitzer H, Smollen JS (1995) Folic acid and cyanocobalamin levels in serum and erythrocytes during low-dose methotrexate therapy of rheumatoid arthritis psoriatic arthritis patients. Clin Exp Rheumatol 13:459–463
19. Ede AE van, Laan RFJM, Blom HJ, Boers GHJ, Haagsma CJ, Thomas CMG, Boo TM de, Putte LBA van de (2002) Homocysteine and folate in methotrexate-treated patients with rheumatoid arthritis. Rheumatology 41:658–665
20. Arthritis and allied conditions (1996) In: Koopman WJ (ed) A textbook of rheumatology. Williams and Wilkins, Baltimore
21. Stabler SP, Marcell PD, Podell EV (1986) Assay of methyl malonic acid in serum of patients with cobalamin deficiency using capillary gas chromatography mass spectrometry. J Clin Invest 77:1606–1612
22. Haagsma CJ, Blom HJ, Riel PLCM van, Hof MA van't, Giesendorf BAJ, Oppenraaij-Emmerzaal D van, Putte LBA van de (1999) Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 58:79–84
23. Yao Y, Yao SL, Yao SS, Yao G, Lou W (1992) Prevalence of vitamin B12 deficiency among geriatric outpatients. J Fam Pract 35:524–525
24. Jensen OK, Rasmussen C, Mollerup F, Christensen PB, Hansen H, Ekelund S, Thulstrup AM (2002). Hyperhomocysteinemia in rheumatoid arthritis: influence of methotrexate treatment and folic acid supplementation. J Rheumatol 29:1615–1618

25. Dharmarajan TS, Norkus EP (2001). Approaches to vitamin B12 deficiency. Early treatment may prevent devastating complications. Postgraduate Med 110:99–105
26. Carmel R (1990). Subtle and atypical cobalamin deficiency states. Am J Hematol 34:108–114
27. Lindenbaum J, Healton EB, Savage DG, Brust JC, Garett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH (1988). Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 318:1720–1728